# Allopatric speciation and multi-trait variation in the common chaffinch (*Fringilla coelebs*) complex

*Master of Science Thesis in Ecology and Evolution*

Even Stensrud


Natural History Museum

University of Oslo

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http://www.duo.uio.no/

Trykk: Reprosentralen, Universitetet i Oslo

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# Abstract

The common chaffinch (*Fringilla coelebs*) is a widespread passerine species in the West Palearctic, and is a common breeder in most of Europe, western Asia, northern Africa, and the Macaronesian islands in the Atlantic Ocean. A total of 19 subspecies are described, whereas five of these are located on the Macaronesian Islands. Three subspecies are found in the Canary Islands, one in Madeira, and one in the Azores. Each of these three archipelagos has a unique climate and ecology, and together with the different geological age and the variation in distance to land these archipelagos provide us with a good background to study evolutionary changes and speciation.

Differences between populations were studied through several traits: plumage differences, biometry, genetic relationships, and differentiation in sperm morphology. All of these traits showed great variation between archipelagos, islands, subspecies, and populations.

The degree of isolation is higher on islands, which might give rise to a larger degree of variation between populations due to drift and selection. The islands subspecies are also generally more differentiated in the studied traits than the populations on the mainland. The sperm measurements show that both the extremes in total sperm length, i.e. the shortest and the longest, are found on the Macaronesian Islands. Also, the variation between archipelagos is greater than the variation between the continental populations, even if the geographical rage on the mainland is of a much greater character. Not surprisingly, the variation among the continental populations is more gradual. Total sperm length, for example, gradually decreases from Morocco to Norway.

The colonization history of the Macaronesian islands is not well understood, but my data support the hypothesis of Marshal and Baker (1999). They argue for a North African origin of the common chaffinch, and that the radiation followed two main routes: one colonizing the Macaronesian islands, while the other spread northwards trough Europe. A Macaronesian colonization from the mainland, to the Azores, trough Madeira, and finally to the Canary Islands seems most likely. Further, the homogeneity on all measured traits among the Azorean populations is in contrast to the large differences between populations in the Canary Islands. Different levels of gene flow among the Azores and the Canary islands can be the origin of these findings.

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# 1 Introduction

The mechanisms behind speciation have intrigued scientists for more than a century and several studies have been conducted to find the important features and driving forces behind diversification and speciation. Allopatric, peripatric, parapatric, and sympatric are four common modes of speciation (Futuyama 2010), where allopatric speciation is widely accepted as the most important speciation force in animals (Mayr 1963; White 1968; Bush 1975). Allopatric speciation occurs when an ancestral population splits in to two daughter population and the gene flow between the two daughter populations is restricted by some kind of barrier. The gene flow between the daughter populations is then absent and the two populations can evolve in different directions by random mutation, drift, and/or selection. When this is retained the two populations are reproductively isolated and genetic differences will accumulate so that the two daughter populations ends up genetically incompatible (Futuyama 2010). Generally, it takes a long time from the two daughter populations split until they reach full reproductive isolation, so in many cases the populations are at an intermediate stage. Just a small amount of gene flow between the sister populations can disrupt the speciation process. Isolated island systems are well known as natural laboratories for evolution and speciation (Gillespie and Roderick 2002; Whittaker 2006). Here, gene flow and immigration will be reduced by the lack of suitable habitat between islands and the continent, and the open sea can be an effective barrier for dispersal. Islands are often small in size and the species richness is low (Futuyama 2010), which provides an unique opportunity to study microevolution, local adaption and speciation.

What is a species? The question has been debated for decades and several species concepts have been suggested. For this study only two of the concepts will be discussed, the biological species concept (BSC) and the phylogenetic species concept (PSC). The morphological species concept dominated for centuries, where species solely were classified based on morphological differences. As species were studied more carefully and new methods were developed, the biological species concept was presented. The biological species concept states that a species is a group of natural interbreeding populations that are reproductively isolated from other such groups (Mayr 1963). The concept is not based on morphology, although most species are morphologically distinct, but rather on reproductive isolation. Species in this concept includes populations that interbreeds or would have the possibility to interbreed without any barriers. One of the problems with the BSC is that it is difficult to directly

observe if geographically isolated populations would interbreed when coexisting. The BSC failure to resolve the pattern and process of taxonomic differentiation and also its emphasis on reproductive isolation gave rise to the phylogenetic species concept. This concept has been revised several times and different definitions exist. In general the PSC recognizes a species as the smallest monophyletic groups of individuals that share at least one unique inherited character (morphological, biochemical, physiological or behavioral). Since species are recognized as diagnosable evolutionary taxa, the reproductive isolation that is prompted in the BSC is eliminated. Thus, two sister taxa can hybridize and still be considered as different species (Cracraft 1983). Proponents of the PSC have in general argued for an elevation of valid subspecies to the rank of species (Cracraft 1983; Davis and Nixon 1992). The BSC have been generally accepted by ornithologists in theory and in practice for half a century. However, during the last two decades the still ongoing debate around species definition, and species concepts have led to a more flexible classification system where other concepts also have an influence. Subspecies is one of the topics that have been debated, since it is widely used in avian taxonomy (Helbig et al. 2002).

The mitochondrial genome of the chicken (*Gallus gallus*) was sequenced as the first contribution to the genetic revolution for the avian taxa (Desjardins and Morais 1990). Since then mitochondrial DNA (mtDNA) has been widely used in phylogenetic avian studies. MtDNA is easily isolated, maternally inherited, and evolves rapidly. Thus, it has given new insight to the study of recently evolved taxa (Avise et al. 1987). Also, evolutionary history, phylogeny, and classification have benefited from this discovery. In the last couple of decades nuclear genes have been used in addition to mtDNA, this to give a more nuanced picture.

Reproductive isolation is a key element in the BSC, and can involve both precopulatory and postcopulatory mechanisms. When two closely related taxa occasionally hybridize the premating barriers are not yet fully established and the barriers could rather be on the postmating level. At this stage of the speciation process, studies of sperm morphology and sperm competition can give new insight to the process, and the mechanisms that establishes reproductive isolation through postmating barriers.

Competition between the sperm of two or more males for a given set of ova can be defined as sperm competition (Parker 1970). This implies that sperm competition might be a powerful form of sexual selection. The early work focused only on the sperm competition between males but later works have shown that cryptic female choice also can have an important

influence on the sperm cells in the female reproductive tract (Eberhard 1996). This makes the system highly complex and the ruling opinion today is that an interaction between the cryptic female choice and sperm competition is important for fertilization (Snook 2005). Many birds exhibit high level of sperm competition despite having a social monogamous mating system (Birkhead and Møller 1992). Normally, sperm competition in birds occurs through extra-pair copulations (EPC), but sperm competition can also arise through rapid mate switching (Birkhead and Møller 1998). Males that engage in multiple mating increase their reproductive output by fathering more offspring than strictly monogamous males (Lank et al. 1989). In species where several males mates with the same female within a short period of time, competition and then selection might occur between the males sperm cells (Birkhead and Møller 1998). Earlier comparative studies have shown that sperm competition leads to more homogeneous sperm size between the males (Calhim et al. 2007; Kleven et al. 2008). This homogeneity is expressed by the coefficient of variation of sperm size between males of each species ($CV_{bm}$). Further, the $CV_{bm}$ measure can be used as a predictor for the species level of sperm competition and further for the species level of extra-pair paternity (EPP) (Lifjeld et al. 2010). Sperm size differences between populations of the same species are still poorly investigated in avian taxa, but the work that have been published on this field shows that there can be substantial differences between populations of the same species (Lüpold et al. 2011; Schmoll and Kleven 2011).

The Macaronesian archipelagos consists of a string of North Atlantic volcanic islands arranged in four archipelagos (the Azores, Madeira, the Canary Islands and the Cape Verde Islands) that emerged 0.4-20 million years ago (Kim et al. 2008). The Islands are spread within a large area (The Azores 1500 km west of Lisbon to Cape Verde 570 km west of Senegal). The large variation in the islands age, size, biota, altitude and latitude as well as the variation of precipitation and the different exposure of water currents makes the Atlantic islands a perfect location to investigate species evolution. The islands are well known for a high rate of endemism (Juan et al. 2000), and are strongly influenced by species from the European mainland. However, there are also a few taxa that originated from the African continent, especially on the southern islands.

The northern Macaronesian avian fauna is in general dominated by species from the European continent, while the Cape Verde Islands have more taxa from the African continent. There are a few endemic bird species on the Macaronesian Islands, like the blue chaffinch (*Fringilla teydea*), which only occurs on Tenerife and Gran Canary. On the other hand, most of the birds

on these islands are endemic subspecies, and in some cases even several endemic subspecies of one species within one archipelago.

The common chaffinch (*Fringilla coelebs*) is a small passerine occurring throughout Europe, northern Africa and the Macaronesian Islands (The Azores, Madeira and the Canaries). Early morphological studies demonstrated that the island common chaffinch have evolved larger body, longer legs and bills, but shorter wings than their continental relatives (Grant 1979). There is also morphological variation between the archipelagos, both in size and plumage. Genetically, the mainland populations are distinct compared with the island subpopulations (Marshall and Baker 1999), and there is variation both within and between archipelagos (Suárez et al. 2009). These findings have created a debate around the phylogeography and the relationship between Macaronesian subspecies, especially on the Canary Islands.

The purpose of this study is to investigate the differences between the populations within the Macaronesian islands, and between the islands and the mainland. To help identify these differences, biometrical measurements, plumage, genetics, and sperm morphology will be investigated. Earlier studies have showed genetic differentiation between the Macaronesian populations and the mainland populations, but also within the Atlantic archipelagos there are clear differences (Dennison and Baker 1991; Marshall and Baker 1999). Since these differences have been shown repeatedly in several studies it is expected that this work will find similar results. The novelty of these analyses is the inclusion of sperm traits. Do sperm traits show a phylogeographic signal to the same extent as genes and morphology? The results from both the sperm data and the genetic data will be compared to uncover possible correlations between genetic distance and sperm morphologic distance. Variation in sperm morphology (e.g. $CV_{bm}$ values) may also reveal patterns in the level of sperm competition and EPP on the island populations. Combining the data on variation in sperm with genetic data, this study can give interesting insights in the possible variation in sperm competition levels between island and mainland populations.

# 2 Materials and methods

## 2.1 Study area

The fieldwork was carried out on the Macaronesian islands in the North Atlantic Ocean over three field seasons. Fieldwork on The Canary Islands was conducted from April to May 2010, from February to May in 2011, on Madeira in April 2011, on the Azores in May 2011 and Gran Canaria in April 2012.

Macaronesia is a collective name for five archipelagos: Azores, Madeira, (including Porto Santo, the Desertas, and the Salvage Islands), the Canary Islands and Cape Verde Islands. The common chaffinch does not occur on the Salvages or the Cape Verde Islands, so these two archipelagoes are not relevant for this study.

The Canary Islands consists of seven main Islands (Lanzarote, Fuerteventura, Gran Canaria, Tenerife, La Gomera, La Palma and El Hierro) and several small associated islets. These islands are close to the African continent and are of relatively recent volcanic origin (table 1), although they also contain the oldest islands within the Macaronesian island complex. There are two dry islands in the east (Lanzarote and Fuerteventura) and five islands ( El Hierro, La Palma, La Gomera, Tenerife, Gran Canaria), in the central and western group that are more humid, and have a larger area with a higher altitude. The same five islands have a wide distribution of laurel forest, together with the endemic canary pine (*Pinus canariensis*) (Lems 1960). The laurel and pine forests are the natural habitat for the chaffinches. The individual islands tend to have distinct microclimates due to their position relative to the north-east trade winds. The westernmost islands (El Hierro, La Palma, and La Gomera) are strongly influenced by the Gulf Stream, and are well vegetated even at low levels, whereas the central islands (Tenerife and Gran Canaria), are less influenced by the Gulf Stream. These islands are drier and closer to the African coast. Fuerteventura and Lanzarote are closest to the African continent and are mainly characterized by desert or semi-desert habitat. The lack of mountains or high altitude areas, together with the high temperature, makes these two western islands uninhabitable for the pine and laurel forest, and then also for the common chaffinch.

Madeira is a volcanic archipelago just 400 km north of Tenerife and is approximately 5 million years old (My). The island's highest point is 1850 meters above sea level and 90 % of the landmass is situated over 500 meters above sea level. The flora is highly divers, with many introduced species (Da Silva Vieira 2002). Large areas of laurel and pine forest make the common chaffinch a common bird on the island. On Madeira, the common chaffinch is mainly found in the forest, (both natal and introduced), but it can also be found in cultivated and rural areas.

The Azores consists of nine major volcanic islands that are clustered in three main groups: The eastern group (Santa Maria and São Miguel), the central group (Terceira, São Jorge, Graciosa, Pico and Faial), and the western group (Flores and Corvo). The archipelago is located 1,500 km west of Lisbon and ranges in age from 8.1 to 0.25 My. The oldest island is Santa Maria and the age generally decreases westwards (except for Pico, which is the youngest island, but situated in the central group). The Archipelago has an oceanic climate characterized by moderate rainfall spread throughout the year, high relative humidity, and small temperature range. The Azores have low floral diversity (approximately 500 species), and many of these species are introduced from western and southern Europe (Haggar et al. 1989; Fernández-Palacios et al. 2011; Connor et al. 2012).

**Table 1.** Overview of: age, size and the geographical placement of the islands in the relevant archipelagos.

| Archepelago | Island | Age | Km² | Coordinates |
|---|---|---|---|---|
| Canary Island | Lanzarote | 25 My | 845 | 29˚02'N, 13˚38'V |
| | Foerteventura | 24 My | 1660 | 28˚24'N, 14˚00'V |
| | Grand Canaria | 15 My | 1560 | 27˚56'N, 15˚36'V |
| | Tenerife | 12 My | 2034 | 28˚17'N, 16˚37'V |
| | La Gomera | 9 My | 369 | 28˚12'N, 17˚12'V |
| | La Palma | 2 My | 708 | 28˚38'N, 17˚51'V |
| | El Hierro | 1 My | 268 | 27˚44'N, 17˚59'V |
| Madeira | Madeira | 5 My | 740 | 32˚45'N, 16˚58'V |
| Azores | Santa Maria | 8.1 My | 37 | 36˚58'N, 25˚06'V |
| | São Miguel | 4.1 My | 293 | 37˚48'N, 25˚28'V |
| | Terceira | 3.5 My | 156 | 38˚43'N, 27˚11'V |
| | Graciosa | 2.5 My | 24 | 39˚03'N, 28˚00'V |
| | Flores | 2.2 My | 55 | 39˚27'N, 31˚11'V |
| | Faial | 0.7 My | 67 | 38˚34'N, 28˚42'V |
| | Corvo | 0.7 My | 7 | 39˚42'N, 31˚06'V |
| | São Jorge | 0.55 My | 95 | 38˚38'N, 28˚03'V |
| | Pico | 0.25 My | 172 | 38˚28'N, 28˚21'V |

## 2.2 Study species

The common chaffinch is a widespread, relatively small passerine in the avian genus *Fringilla*. The males have a rust colored face with a black forehead, rusty colored breast and belly, and black wings with white on outer tail feathers. Females have the same facial features, but they are generally paler with a brown-olive plumage.

The *Fringilla* genus consists of three species: The brambling (*Fringilla montifringilla*), the blue chaffinch *(Fringilla teydea*), and the common chaffinch. The brambling is a widespread bird throughout the boreal forests of northern Europe and Asia. The blue chaffinch is endemic to the Canary Islands and divided into two subspecies (*F. t. teydea* on Tenerife and *F. t. polatzeki* on Gran Canaria). The common chaffinch is well known throughout Europe, but its range extends into western Asia, northwestern Africa, and several of the Macaronesian islands. The common chaffinch complex consists of at least 19 described subspecies (Gill and Donsker 2012). In the Macaronesian islands there are described 5 subspecies, hereafter ssp., of the common chaffinch. In the Canary Islands there are three: *ssp. canariensis* on Gran Canaria, Tenerife and La Gomera, *ssp. palmae* on La Palma, and *ssp. ombriosa* on El Hierro. In Madeira there is one subspecies: *ssp. madeirensis* and on the Azores we find the *ssp. moreletti*.

The three subspecies described on the Canary Islands are mainly based on morphological traits, and this subdivision has given rise to decades of debate. Studies based on electrophoretic mobility of proteins suggested only two subspecies, where the subspecies on the two western islands (El Hierro and La Palma) should be merged to one (Baker et al. 1990). This result is consistent with an early morphometric study (Grant 1979). Later studies have included mtDNA markers (Marshall and Baker 1999) and microsatellites (Suárez et al. 2009) to further investigate the phylogenetic relationships. The latter study showed that there is likely to be at least three subspecies on the Canary Islands, but with a different distribution than has been morphologically accepted. Suarez et al. (2009) also suggests that El Hierro and La Palma should be treated as one ssp., while Gran Canaria should be split from Tenerife and La Gomera (ssp. *canariensis*) and treated as a genetic distinct taxonomic unit. These different results show the need for a taxonomic re-evaluation of the common chaffinch populations and subspecies on the Canary Islands.

Phenotypically, the different subspecies on the Macaronesian Islands are different from both the European and the North African subspecies (Grant 1979). The easy recognizable brown

back on the European subspecies is lacking on the Macaronesian and African birds, and is replaced with a green to olive green color. Further, the white eye-ring makes the African birds easy to distinguish from the others. Biometrical measurements also show a variation between mainland and Island birds (Grant 1979). In general, the Macaronesian birds are heavier, have shorter wings, and a bigger beak than the mainland subspecies.

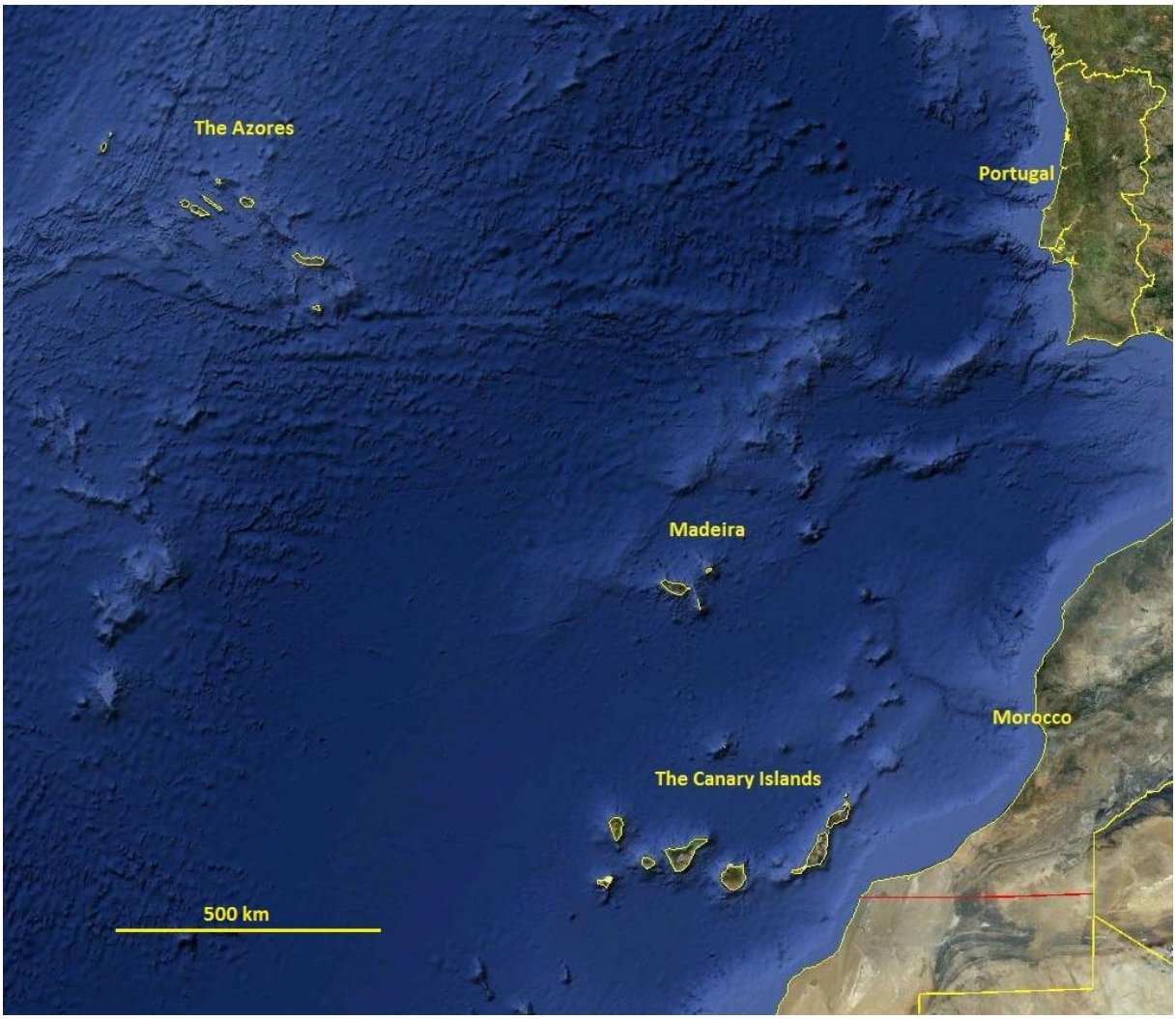


**Figure 2.1.** Map showing all the Macaronesian islands where the common chaffinch occurs.


**Figure 2.2.** Map showing the Canary Islands and Madeira.

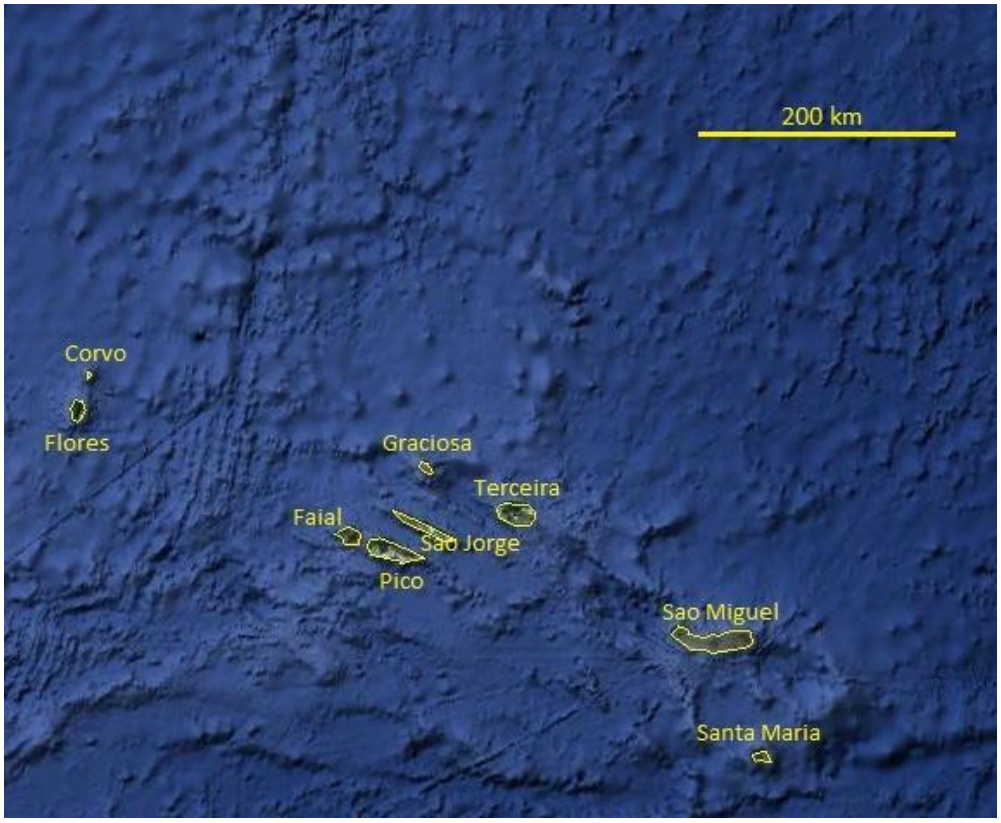


**Figure 2.3.** Map showing the Azorean Islands.

## 2.3 Field procedures

Adult males were generally caught with mist nets and playback, whereas some of the males were caught at picnic sites using clap nets baited with seeds or bread. Biometric measurements (wing length, tarsus length and mass) were taken and adult males where photographed. Blood and sperm samples were collected (see below for procedures) from all the males before they were banded with unique aluminum bands and released in same area as they were caught. The fieldwork was conducted from February until June, which corresponds to the period in which breeding occurs and males are sexually active. This long sampling period was also influenced by the variation in reproductive timing between the populations.

## 2.4 Plumage and biometry

All the birds that were caught during fieldwork were photographed and measured. Two pictures of representative adult males from each population were chosen. The plumage differences between populations were not tested in any way based on the pictures; the assembly only gives an impression of the variation. The pictures illustrates that there are variation in the coloration between populations within the chaffinch complex. The measurements were mainly done by one person (T. Laskemoen) to exclude measurement bias, whereas the birds from the Azores were measured by a second person (P. Rodrigues). The same method and equipment was used for wing and mass measurement, whereas the tarsus length was measured with a different technique and the Azorean birds have thus been excluded for this trait. For wing length, flattened and straightened method was used (Svensson 1992). For the tarsus length measurement the maximum tarsus method were used. The weight was measured with a handheld spiral balance (Pesola). Only measurements of adult males were used in further analysis.

## 2.5 Genetic analyses

### 2.5.1 Sampling

Blood samples were taken from all the males by venipuncture of the ulnar vein and preserved in absolute alcohol until DNA extraction. The blood samples in the present study are from birds caught in the period from 2007 to 2012. For this molecular work, 3- 10 samples per population were used, a total of 105 samples. Out of these 105 samples, 30 are downloaded from Barcoding of Life Data Systems (BOLD) (Ratnasingham and Hebert 2007), (16 *Fringilla montifringilla* and 14 *Fringilla coelebs*) (appendix 1). DNA was extracted from blood stored on ethanol using a commercial kit (E.Z.N.A. DNA Kit; Omega Bio-Tek, Norcross, GA, USA) following the manufacturer's specifications. DNA was extracted using a commercial spin column kit (E.Z.N.A. DNA Kit; Omega Bio-Tek, Norcross, GA, USA) or a GeneMole® automated nucleic acid extraction instrument (Mole Genetics), following the manufacturers' protocols.

### 2.5.2 DNA extraction, PCR amplification and sequencing

DNA extraction, PCR amplification, and DNA sequencing was performed at the Natural History Museum in Oslo. A 655 base pair (bp) fragment of the 5'-end of cytochrome c oxidase I (CO1) gene was amplified and sequenced using the primers PasserF1 CCAACCACAAAGACATCGGAACC and PasserR1 GTAAACTTCTGGGTGACCAAAGAA TC (Lohman et al. 2009) for most individuals. For some individuals BirdF1 TTCTCCAACCACAAAGACATTGGCAC (Hebert et al. 2004) or CO1-ExtF ACGCTTTAACACTCAGCCATCTTACC (Johnsen et al. 2010) and BirdR2 ACTACATGTGAGATGATTCCGAATCCAG (Hebert et al. 2004) were used. A 1009 bp long fragment of the cytochrome b (cytb) gene was amplified using the primers L14841 AAAAAGCTTCCATCCAACATCTCAGCATGATGAAA (Kocher et al. 1989) and H15915 CTCTTCAGTTTTTGGTTTACAAGAC (Fu 2000). For the control region a 507 bp long fragment was amplified using the primers FCR_15 TCAGGGTATGTATAATATGC and FCR_13 CACTTGCTGTGAAGAGC (Baker and Marshall 1997).

PCR reaction volumes were 12.5µl, containing 0.5mM dNTPs, 0.025 U/µL Platinum *Taq* DNA polymerase (Life technologies), 1 x PCR buffer, 2.5 mM $MgCl_2$, 0.1 mM of each primer and 2 µL DNA extract. The cycle-sequencing reactions were carried out using the ABI PRISM BigDye Terminator v3.1 Cycle Sequencing Kit, and run on an ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems) following the manufacturer's instructions.

The PCR conditions for CO1 were as follows: 94 ˚C for 2 min, 35 cycles of (94 ˚C in 30s, 55 ˚C (50 ˚C) for 30s, 72 ˚C for 45s), 72 ˚C for 7 min. The PCR and sequencing products were cleaned with ethanol precipitation. For cytb and the control region the only deviation from this manual was that the annealing temperature was 55 ˚C instead of 50 ˚C.

All sequences are deposited in BOLD (Ratnasingham and Hebert 2007) and GenBank (see Appendix 1).

### 2.5.3 Phylogenetic analyses

All sequences were edited and aligned using ClustalW (Thompson et al. 1994) in CodonCode Aligner 3.7.1. (CodonCode Corporation). Neighbor-joining analysis was calculated in MEGA 5 using the Kimura 2-parameter (K2P) algorithm (Tamura et al. 2011) with all sites included, the complete deletion option, assuming homogenous pattern among linages and uniform substitution rate among sites. The concatenated tree neighbor-joining analyses were used after the three sequences where concatenated, aligned and partitioned.

The median-joining haplotype network where constructed in NETWORK 4.6.1.0 (available at http://www.fluxustechnology.com/).

### 2.5.4 Sperm measurements

Sperm samples were collected by gently massaging the cloacal protuberance of the bird (Wolfson 1952; Kleven et al. 2008). The sperm samples were stored in a 5% formalin solution. Approximately 10 ml of sperm were applied to microscope slides using a pipette. The slides where dried before they were washed with distilled water to remove salt crystals. The equipment used for the analyses of the samples was a Zeiss AxioCam camera mounted on a Zeiss Axioplan 2 light microscope to take digital photographs of spermatozoa at a magnification 200 or 400 ×. Pictures of ten normal sperm from each individual were taken and then the components were measured with Zeiss AxioVision 4.8. Three components were

measured per cell: head, midpiece and tail. The total length was calculated by adding the lengths of these three components. Sperm cells that were abnormal, broken or in any other way difficult to measure were discarded. Passerine sperm are characterized by having a helical shaped head (acrosome + nuclear region) and a straight flagellum (midpiece + tail), or both helical shaped head and flagellum (Koehler 1995). Common chaffinch sperm are characterized as the latter (figure 2.4). Also, Common chaffinch sperm are relatively long compared with other finches (table 4.1). In addition to the samples collected during the three field seasons, samples from the sperm collection at the Natural History Museum, University of Oslo, were included. These samples originated from Norway, Morocco, Ukraine and Spain. In total, 234 individuals were measured of which 177 were from the Macaronesian islands and 57 from mainland populations.

As a standardized measure of variation, the coefficient of variation (CV = (SD/mean)×100) were used, denoted as $CV_{bm}$ for the between-male CV in mean sperm length and $CV_{wm}$ for the mean within-male CV in sperm length. This was done for all males inn all populations (table 2). CVbm values have been shown as strong predictors for the rate of extra pair paternity (EPP) (Lifjeld et al. 2010) and might indicate differences between the populations in the Macaronesian islands since EPP data on this populations so far does not exist. Notably, the CV of small samples tends to be underestimated (Sokal and Rohlf 1995; Laskemoen et al. 2007). Hence, I adjusted for this following the formula: $CV_{adj}=CV\times(1+(1/4n))$ (Sokal and Rohlf 1995).

The mean values of each component and of the total length were calculated for each male. All statistical analyses were performed using R version 2.11.1 (R Development Core Team 2011) software package, and the box plots were constructed in Origin version 7.0300.

A correlation between sperm morphologic distance and genetic distance among pairs of populations was calculated. The genetic distance matrix was calculated in Mega, using the Kimura 2-parameter algorithm (K2P) (Tamura et al. 2011) with all sites included, the complete deletion option, assuming homogenous pattern among linages, and uniform substitution rate among sites. Values in the sperm distance matrix represent the absolute differences in the total sperm length between pairs of all populations. Five populations from the Canary Islands are included together with birds from Madeira. A Mantel test was used for the correlation, since it corrects for multiple testing of populations.

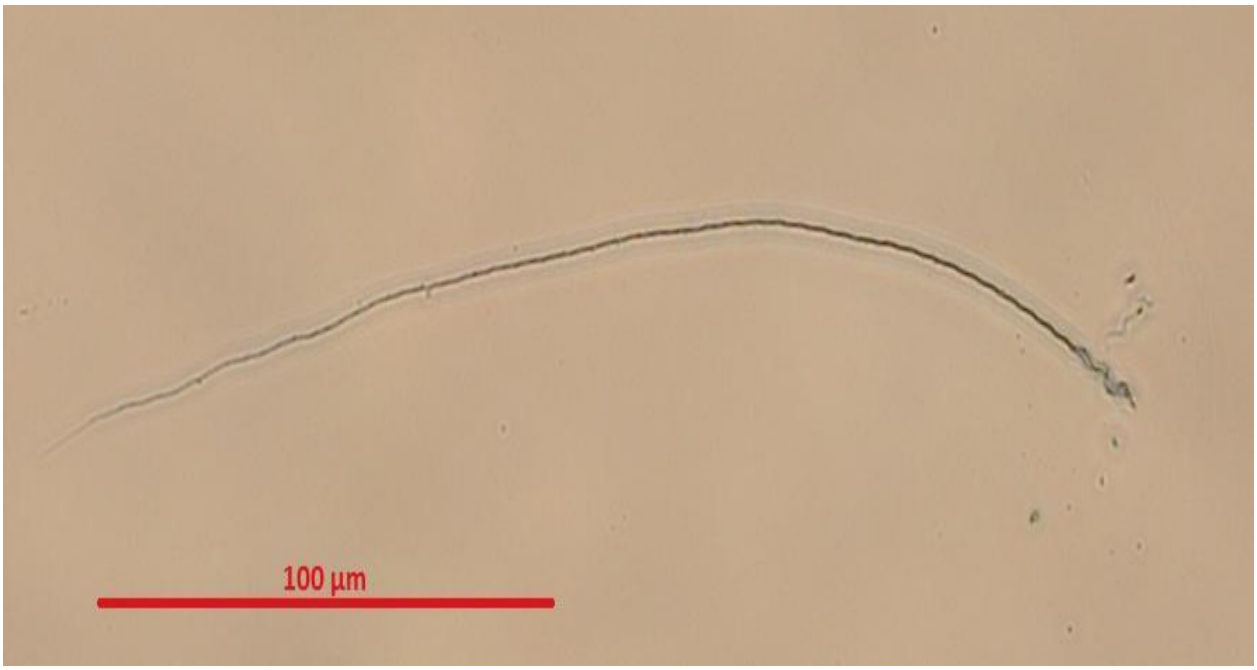


**Figure 2.4.** Representative sperm cell from a common chaffinch (*Fringilla coelebs*).

# 3 Results

## 3.1 Plumage variation

There is large variation in plumage among males within the Macaronesian populations. Especially the coloration on the mantle and back is substantial. To sum up the most important differences, the coloration on variable parts of the bird`s plumage was scored from the photos (Fig. 3.1 & 3.2) and given a representative color (table 4). Photos have been subjectively chosen to illustrate the most representative male phenotype from each island. Ssp. *canariensis* has an all blue crown, mantle and back. The rump is bright green. The cheeks and chest are light orange. This color is generally fading from the chest to the vent, but in some individuals the coloration is present all the way from the cheeks to the vent. The beak is steel blue, and there is some amount of green in the tail and wing feathers. The black band on the forehead is present. The two most variable traits between populations are the intensity of the orange color on the chest and the amount of green on the back. Even though the population on Gran Canaria and Tenerife belongs to the same subspecies (together with La Gomera), they are both present in the assembly because there might be some minor differences in the amount of green on the rump. They are scored equally in the table and are considered as one subspecies in the text. Ssp. *ombriosa* (El Hierro) shows similar coloration patterns but the amount of green on the back is more restricted and the coloration on the chest is paler and has a gray color from the breast and down. Ssp. *palmae* is strikingly blue. The amount of green on their back, tail and wing is almost non-existing. The light orange/rust color is restricted to a small area around the beak, and the rest of the chest is gray, and in some adult males it is close to white. The Madeira subspecies*:* ssp. *madeirensis*, has the same amount of green on the rump as the ssp. *canariensis*, but in addition has a green band further up on the back around the backside of the neck. The cheeks and upper part of the breast have the orange/rust, color but also here the color fades out on the way backwards and gives a gray impression from the lower part of the chest and down to the vent. The green in the tail and wing feather is present and the beak is darker blue. The subspecies form the Azores, ssp. *moreletti*, has a large orange/rust colored area on the cheeks and breast like the ssp. *canariensis*. The back is all green even though the coloration might be spotted and in some cases also interspersed with some brown feathers. The green on the tail and wing feathers resembles ssp. *madeirensis*. The

blue areas on the head and back on ssp. *madeirensis* and ssp. *moreletti* are of a darker character and gives a grayer impression than the subspecies from the Canary Islands. The ssp. *coelebs* gives a quite different impression, with the dark brown back and the bright orange coloration on the cheeks and breast. The crown and mantle are closer to gray and the vent has a pink color. Also the continental birds have a bright green rump. The North-African subspecies ssp. *africana* shows some unique features, the cheeks are all grey/ blue and the orange/ pink color is restricted to the throat and breast. The color is fading into light gray on the vent. The back is all bright green but can be spotted like ssp. *moreletti.* The black forehead is present and they have a characteristic white eye ring.

**Table 2.** The table illustrates the different subspecies variation in color based on a subjective scoring.

| Subspecies | forehead | crown | mantle | back | rump | cheek | breast | vent |
|---|---|---|---|---|---|---|---|---|
| *canariensis GC* | | | | | | | | |
| *canariensis TEN* | | | | | | | | |
| *palma* | | | | | | | | |
| *ombriosa* | | | | | | | | |
| *madeirensis* | | | | | | | | |
| *moreletti* | | | | | | | | |
| *coelebs* | | | | | | | | |
| *africana* | | | | | | | | |

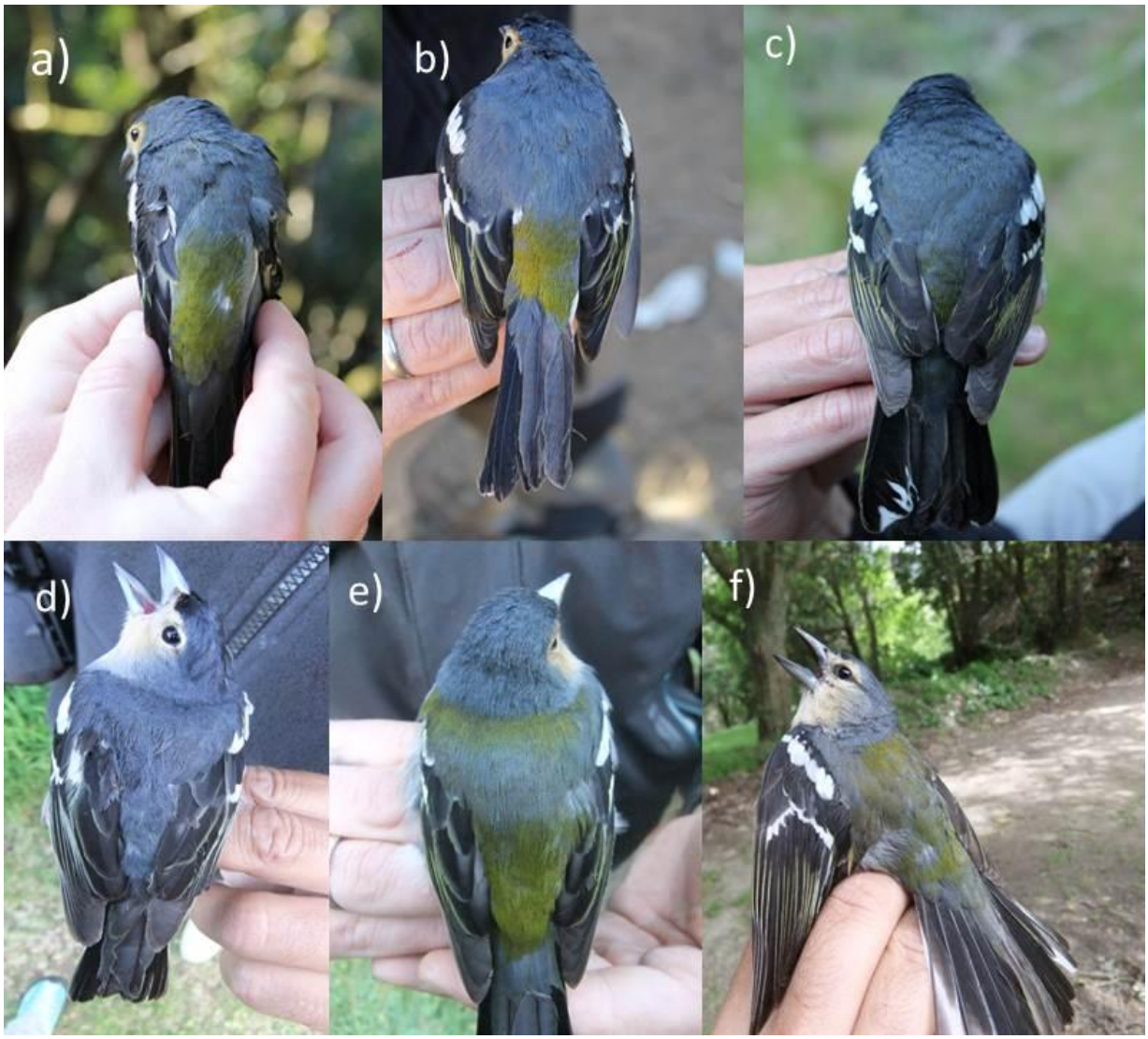


**Figure 3.1.** Back view of representative males from each island; a) ssp. *canariensis* from La Gomera, b) ssp. *canariensis* from Gran Canaria, c) ssp. *ombriosa* from El Hierro, d) ssp. *palmae* from La Palma, e) ssp. *madeirensis* from Madeira and f) ssp. *moreletti* from the Azores. The population from Tenerife is not present in this collage because they resemble the La Gomera population. Photos taken by: J. Gohli, T. Laskemoen and E. Stensrud.


**Figure 3.2.** Side view of representative males from each island; a) ssp. *canariensis* from La Gomera, b) ssp. *canariensis* from Gran Canaria, c) ssp. *ombriosa* from El Hierro, d) ssp. *palmae* from La Palma, e) ssp. *madeirensis* from Madeira and f) ssp. *moreletti* from the Azores. The population from Tenerife is not present in this collage because they resemble the La Gomera population. Photos taken by: J. Gohli, T. Laskemoen and E. Stensrud.


**Figure 3.3.** Side and back view of representative males. g) ssp. *coelebs* from Norway, h) ssp. *africana* from Morocco. Photos taken by J.L. Copete, L.E. Johannessen and T. Laskemoen.

## 3.2 Biometry.

Variation in biometric traits is presented in table 3. Wing length differed significantly among populations (ANOVA; $p<0.001$). Within the Macaronesian islands, birds from Gran Canaria have the shortest wings, while birds from La Gomera have the longest. The Norwegian birds have the longest wings of all measurements. Tarsus length differed significantly between populations (ANOVA; $p<0.001$). The Macaronesian birds have longer tarsi than the Norwegian. The Gran Canarian populations have the shortest tarsi of the island birds, and La Gomera the longest. Body mass differed significantly between populations (ANOVA; $p<0.001$). Norwegian and Gran Canarian populations are the lightest, while the birds from La Gomera also on this trait are the biggest.

In summary, the biometric results show that there are significant variations between populations on all traits. Within the islands, the birds from Gran Canaria are the smallest on all traits while the birds from La Gomera are the largest/heaviest. The birds from the Norwegian population have longer wings than the Macaronesian birds but their tarsi are short and their body mass are among the lowest (table 3). All measurements are from adult males.

**Table 3.** Overview of the biometric measurements, and test statistics for eight populations of common chaffinch (*Fringilla coelebs*). All measurements are from adult males.

| Location | Wing length (mm) | Tarsus length* (mm) | Body mass (g) | N[$] |
|---|---|---|---|---|
| Gran Canaria | 82.8 ± 1.9 | 24.2 ± 0.7 | 22.2 ± 1.0 | 19/19/13 |
| Tenerife | 86.2 ± 2.3 | 24.6 ± 0.7 | 24.5 ± 1.3 | 12/12/10 |
| La Gomera | 88.1 ± 2.9 | 25.5 ± 0.7 | 26.7 ± 1.2 | 11/6/11 |
| El Hierro | 87.4 ± 2.3 | 25.1 ± 0.5 | 25.9 ± 1.3 | 17/17/6 |
| La Palma | 88.3 ± 2.1 | 24.6 ± 0.7 | 27.3 ± 1.4 | 26/26/14 |
| Madeira | 85.5 ± 1.9 | 24.8 ± 0.7 | 23.5 ± 1.3 | 41/40/32 |
| Sao Miguel* | 85.1 ± 2.0 | - | 26.5 ± 0.7 | 8/-/8 |
| Norway | 89.3 ± 2.5 | 21.1 ± 0.8 | 23.3 ± 1.6 | 19/14/6 |
| [¤]*F*-values | $F_{7,145}$= 17.766 | $F_{6,127}$= 63.575 | $F_{7,92}$= 44.797 | |
| *P*-values | < 0.001 | < 0.001 | < 0.001 | |

*The birds from Sao Miguel (Azores) were measured by a second person, using a different technique. Thus, the tarsus values for this population were not comparable to the rest. [$]Sample sizes for wing length, tarsus length, and body mass respectively. [¤]*F*-values from ANOVA.

## 3.3 Phylogenetic structure

The phylogenetic analyses were based on a total of 105 individuals. It includes all the common chaffinches together with the brambling (*Fringilla montifringilla*) and the blue chaffinch (*Fringilla teydea*). The neighbor-joining tree is the result of analyses made from a 655 bp fragment of the CO1 gene (figure 3.4). Both the brambling and the blue chaffinch are out groups to the common chaffinch complex, were the blue chaffinch is the closest relative. For the common chaffinch complex, the phylogeny first splits into two clades. One clade consists of the mainland individuals from Morocco, Norway, Tunisia, and the rest of the European populations. The other clade is the Atlantic clade where all the subpopulations from the Macaronesian islands are found. All the subspecies and populations from the Macaronesian islands fall in to this clade. Further the Atlantic clade splits repeatedly, first the ssp. *moreletti* from the Azores are divided from the ssp. *madeirensis* and the Canary Island populations. The Azorean birds are divided into two groups with a relatively deep split. Both haplotypes are mixed among all islands without any clear geographic pattern. This might imply that two separate colonization events created the two haplotypes. The ssp. *madeirensis* differentiate first, while the Canary Island populations are placed together. The population on Gran Canaria comes out alone and separated from the two other populations within the ssp. *canariensis* (Tenerife and La Gomera). The two ssp. palmae and ombriosa are clustered together and cannot be separated on the CO1 gene. The mainland clade divides into two groups; one with only Moroccan samples and one with all the others. That is the rest of the Moroccan samples together with the other North African population from Tunisia. European individuals from Spain, Russia, Sweden, and Norway come out here together with one interesting sample from the island of Faial in the Azores. Because of low bootstrap values for the continental branch, they are collapsed into one clade. Another interesting feature is that one individual from Tunisia is placed as a sister group to the Macaronesian clade in the CO1 tree, but as a sister to the whole common chaffinch complex in the concatenated tree. Similar patterns have been described earlier for the ssp. *spodiogenys* (Marshall and Baker 1999). The bootstrap values for the placement of this individual are low, but it is still unique.

Generally, the phylogeny shows high node support, but to investigate the topology further and to rule out the possibility of pseudo genes, a concatenated neighbor-joining tree was made (figure 3.5). Cytb and control region sequences where added to the CO1 sequences for this

analyses. The concatenated tree consists of carefully picked individuals that represent each clade in the CO1 tree. The concatenated tree manages to separate the populations from El Hierro (ssp. *ombriosa*) and La Palma (ssp. *palmae*) and the structure within the continental clade gets higher bootstrap values. Apart from this, the two trees are almost identical. Also this analysis has generally high support values.

The haplotype network based on the CO1 sequences was created to illustrate the split and distance between the continental and the Atlantic clade (figure 3.6). The Atlantic clade shows the same division as expected from the earlier trees. Two common haplotypes is found on the Azores, the third on contains two individuals from Santa Maria (appendix 2). El Hierro and La Palma are identical, Tenerife and La Gomera situates together and Gran Canaria is unique and separated from the other ssp. *canariensis* populations. The Atlantic clade is clearly differentiated from the mainland populations with the two pure Moroccan haplotypes in between. The mainland clade consists of nine haplotypes where the common haplotypes are shared between different populations. The birds from Tunisia have four unique haplotypes, whereof one of these also here shows a large differentiation from the rest (Tunisia_38941).

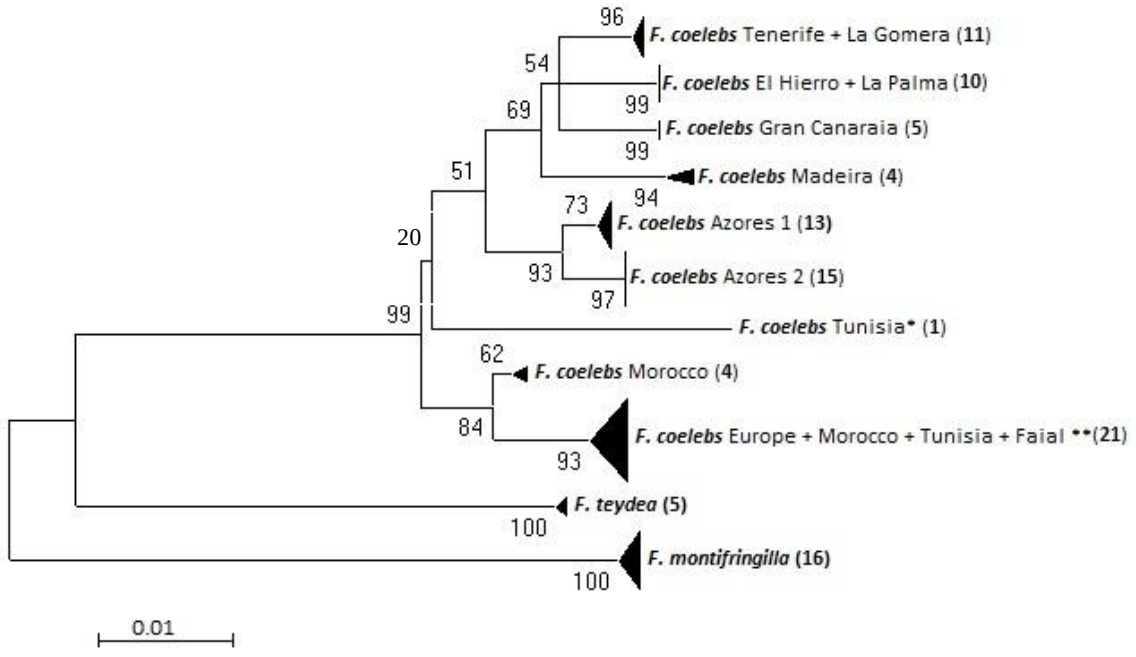


**Figure 3.4.** Tree topology and bootstrap confidence levels obtained from neighbor-joining analyses for all the CO1 sequences. *One Tunisian sample (38941). ** One sample from Faial (Azores) within the mainland clade. The black triangles represent the sample size and the amount of internal structure.

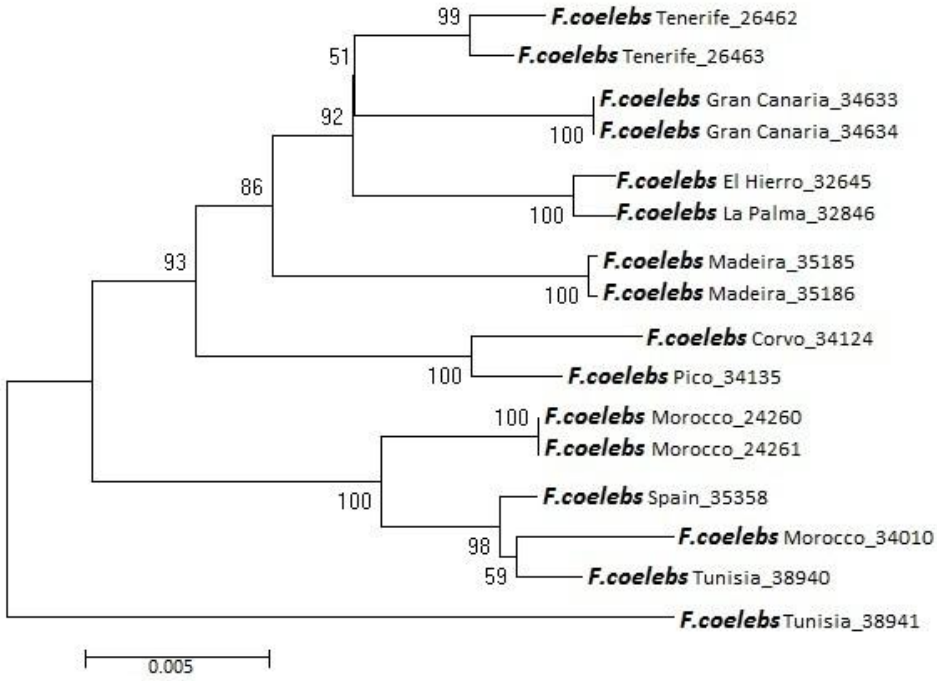


**Figure 3.5**. Tree topology and bootstrap confidence levels obtained from neighbor-joining analyses for the concatenated sequences (CO1, cytb and the control region).

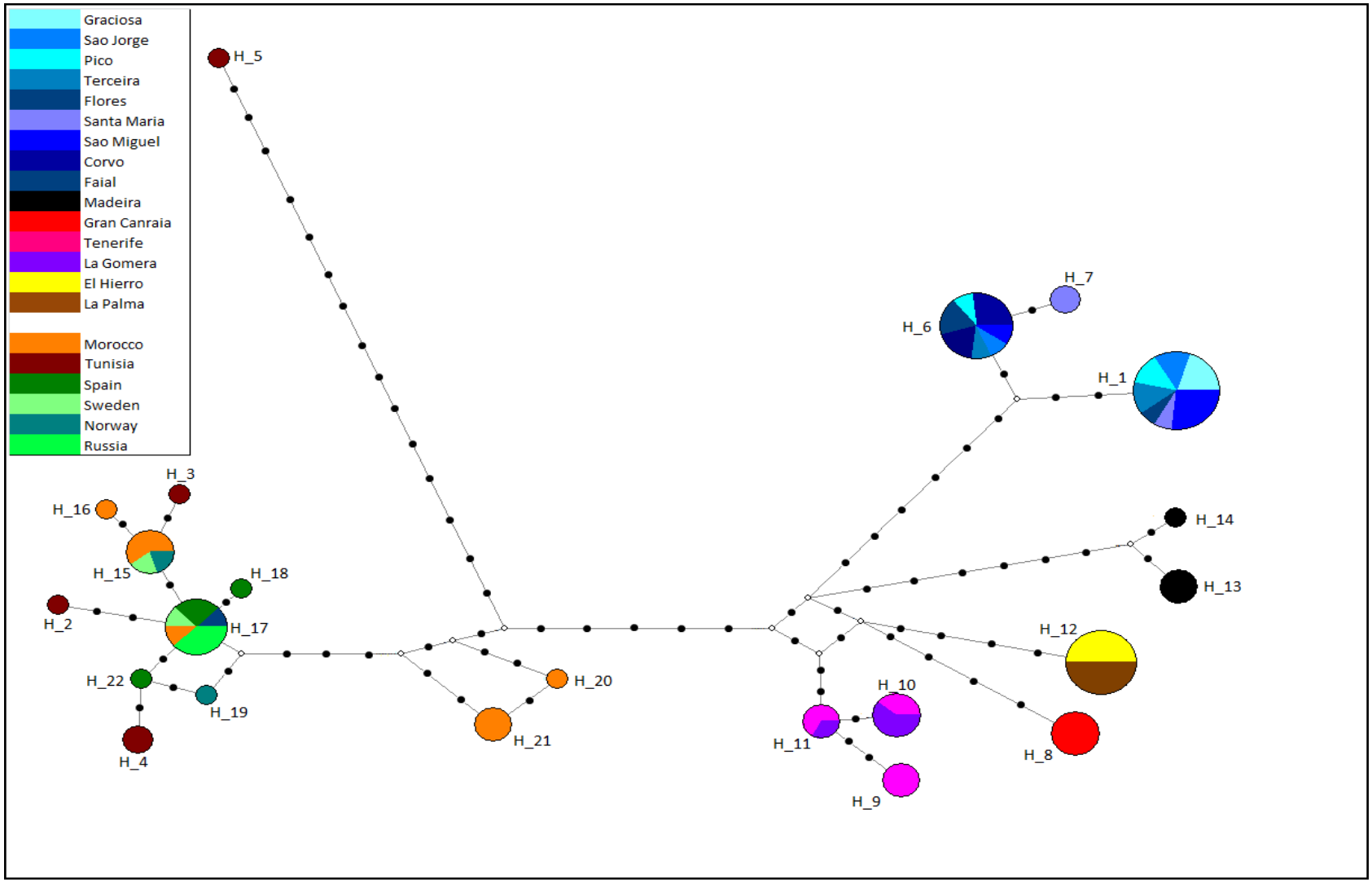


**Figure 3.6.** Median-joining haplotype network from CO1 sequences. Each circle represents a haplotype with its size proportional to its frequency. Black dots on the line connecting two haplotypes represent mutational steps between haplotype

## 3.4 Sperm measurements

Descriptive data on all sperm traits are presented in Table 4. There is significant variation in all sperm traits among all populations (ANOVA: head: $F_{17,211}$ =10.68, p<0.001 ; midpiece: $_{F17,211}$ =28.221, p<0.001 ; tail: $F_{17,211}$=2.424, p=0.00181 ; total: $F_{17,211}$=39.147, p<0.001). The boxplot (fig.10) illustrates the variation in total sperm length between the different populations. The total sperm length clearly divides into two groups where the birds from Canary Islands have shorter sperm than the birds from Madeira, The Azores and the mainland populations. Canary Islands and Madeira are significantly different in total sperm length (ANOVA; $F_{5,64}$ = p< 0.001). The variation within the Canary Islands is extensive, with Gran Canaria significantly different from all the other islands (pairwise t-test; p < 0.001). The other Canary Islands are also different from each other, except from Tenerife and La Gomera, and La Gomera and El Hierro (appendix 3). The variation within the Azores is limited and there is no significant variation within the archipelago for total sperm length. For the mainland populations the total sperm size is declining with latitude, the northern individuals have shorter sperm cells than southern individuals. The Birds from Morocco have the longest sperm cells while the Norwegian population has the shortest. Generally, sperm head length show minor differences between populations. The tail is slightly more variable, but it is the midpiece that generates most of the variation in length between different sperm cells (figure 3.8). Ten measured cells per individual is enough to give a good estimate of the individuals average sperm size, but to give a satisfying estimate of the individuals variation ($CV_{wm}$) this number should have been doubled (Laskemoen et al. 2007). However, as indicated in table 4, the $CV_{wm}$ values are only slightly variable between populations and might indicate that the sperm size variation within individuals is low. The $CV_{bm}$ values show some variation among populations, but all values are low and might imply that the common chaffinch is among the species with high sperm competition. For some of the islands and populations the sample size is low, but generally the sample size is large enough to give a good estimate of the populations $CV_{bm}$.

The Mantel test indicates a positive correlation between the sperm morphologic distance and the genetic distance (figure 3.9). The Monte Carlo simulations rejected the Mantel tests null hypotheses of no relationship (p=0.023) and the observed value is 0.66. This result implies that populations with large genetic difference, it is likely to also have large sperm morphologic difference.

**Table 4.** Summary of all sperm measurements grouped by populations.

| Location | N | Head | midpiece | Tail | Total | $CV_{bm}$^ | $CV_{wm}$* |
|---|---|---|---|---|---|---|---|
| Gran Canaria | 15 | 15.74 ± 0,47 | 202.17 ± 3.63 | 10.05 ± 3.20 | 227.96 ± 3.85 | 1.72 | 1.49 |
| Tenerife | 12 | 15.86 ± 0,63 | 221.91 ± 5.54 | 10.58 ± 2.02 | 248.36 ± 4.71 | 1.94 | 1.29 |
| La Gomera | 9 | 15.82 ± 0,71 | 218.47 ± 2.75 | 8.81 ± 1.78 | 243.10 ± 2.31 | 0.98 | 1.44 |
| La Palma | 13 | 15.84 ± 0,69 | 209.36 ± 5.15 | 8.80 ± 1.83 | 233.99 ± 3.99 | 1.74 | 1.66 |
| El Hierro | 13 | 16.03 ± 0,47 | 214.32 ± 4.74 | 9.57 ± 2.21 | 239.92 ± 5.95 | 2.53 | 1.50 |
| Madeira | 8 | 15.83 ± 0,53 | 231.97 ± 8.56 | 13.65 ± 6.91 | 261.45 ± 5,51 | 2.18 | 1.53 |
| Santa Maria | 10 | 15.95 ± 0,74 | 228.29 ± 2.50 | 11.82 ± 3.03 | 256.06 ± 3,31 | 1.32 | 1.50 |
| Terceira | 24 | 16.17 ± 0,59 | 225.31 ± 5.77 | 11.39 ± 3.22 | 252.87 ± 4,9 | 1.96 | 1.42 |
| Sao Jorge | 15 | 16.04 ± 0,65 | 228.59 ± 2.56 | 9.27 ± 2.28 | 253.89 ± 3,96 | 1.59 | 1.20 |
| Sao Miguel | 23 | 15.66 ± 0,75 | 221.13 ± 7.19 | 13.50 ± 4.78 | 250.30 ± 5,15 | 2.08 | 1.63 |
| Faial | 7 | 15.62 ± 0,65 | 225.35 ± 5.62 | 11.37 ± 1.89 | 252.34 ± 6,17 | 2.53 | 1.17 |
| Flores | 19 | 16.08 ± 0,68 | 221.44 ± 6.13 | 13.90 ± 6.34 | 251.42 ± 4,51 | 1.81 | 1.50 |
| Pico | 4 | 15.83 ± 1,21 | 223.64 ± 7.44 | 10.45 ± 1.82 | 249.92 ± 6,54 | 2.78 | 1.25 |
| Corvo | 3 | 16.42 ± 0,63 | 229.31 ± 2.52 | 11.15 ± 1.00 | 256.88 ± 2,82 | 1.19 | 1.36 |
| Morocco | 6 | 15.70 ± 0,62 | 238.12 ± 2.07 | 8.99 ± 1.51 | 262.81 ± 3,04 | 1.21 | 1.21 |
| Norway | 39 | 16.12 ± 1,25 | 225.20 ± 6.53 | 10.66 ± 3.63 | 251.98 ± 5,5 | 2.19 | 1.48 |
| Spain | 6 | 14.40 ± 0,91 | 231.47 ± 6.11 | 10.62 ± 1.77 | 256.50 ± 6,54 | 2.68 | 1.48 |
| Ukraine | 5 | 13.60 ± 0,56 | 231.69 ± 1.76 | 10.45 ± 1.81 | 255.74 ± 3,02 | 1.24 | 0.92 |

^CVbm adjusted for sample size.

*avg. within-male CV

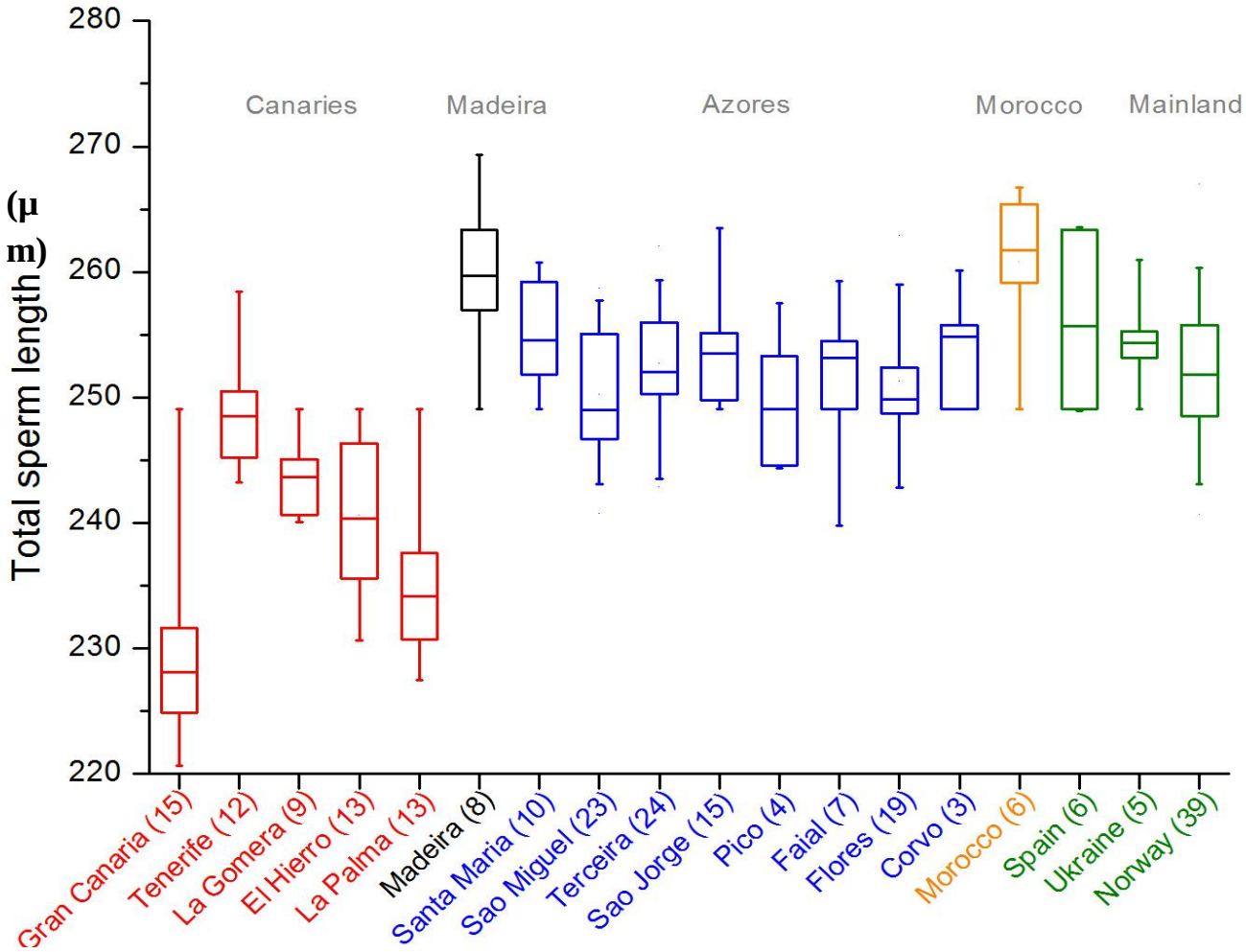


**Figure 3.7.** The sperm measurements are grouped by populations, not subspecies since there is variation within the subspecies. The boxes illustrate median and quartiles.

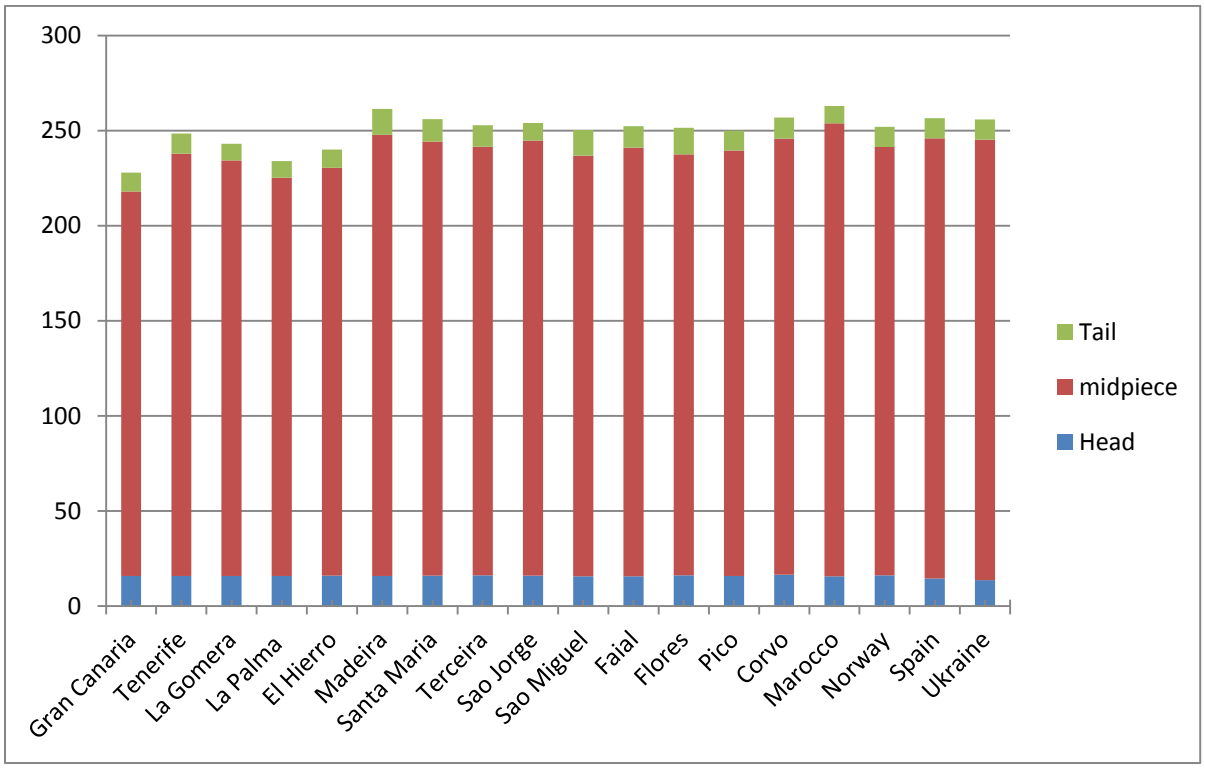


**Figure 3.8**. The figure illustrates the components contribution to the total sperm length. Values are given in µm.

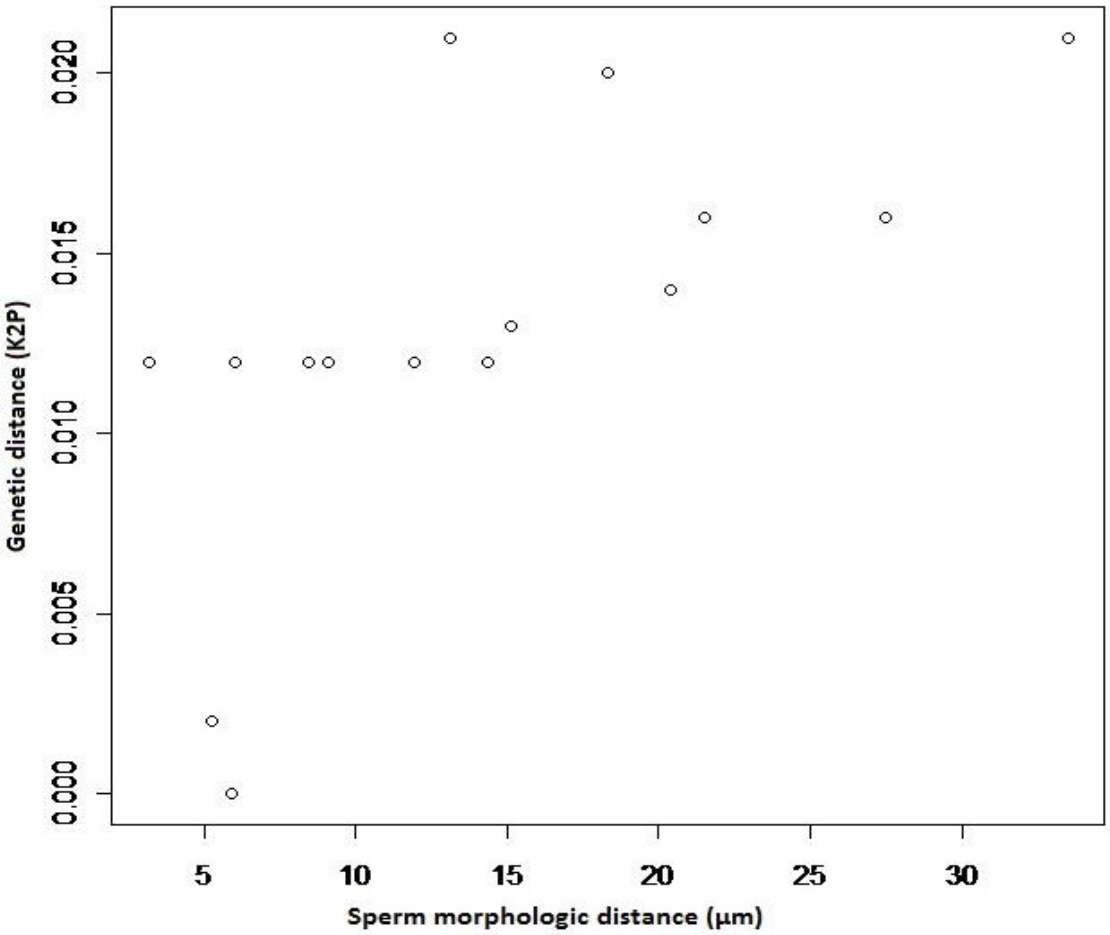


**Figure 3.9.** The figure illustrates the relationship between the sperm morphologic distance and the genetic distance. See Results for test statistics.

# 4 Discussion

The variation between subspecies and population within the common chaffinch complex is extensive. The results from this study show that there is variation on all measured traits between subspecies, but also between populations. The differences between the Macaronesian islands and the continental populations are striking. Not only the easy recognizable plumage differences or biometric differences divide these two groups; also the genetic analyzes and the sperm morphological measurements show a differentiation. The Macaronesian archipelagos show interesting variation, where the homogeneity among the Azorean islands is in contrast to the large variation among the Canary island populations.

Island systems have intrigued scientist for centuries (MacArthur and Wilson 1967). Isolated islands are the perfect scenery for observing ongoing allopatric speciation and evolution. One of the most famous examples of island differentiation and speciation is the Galapagos Islands, where a monophyletic group of 13 bird species, i.e. the Galapagos finches, have been studied for decades (Grant 1999). An ancient group of birds colonized the Galapagos Islands approximately 2.3 million years ago (Sato et al. 2001). This is more than twice as long as the estimate for the common chaffinches' colonization of the Macaronesian archipelagos (Suárez et al. 2009). There is large variation in morphology and biometry between the species on the Galapagos Islands, while the genetic distances between the species is not extensive (Sato et al. 1999). The beak size is the most variable trait among the species, and studies have shown that beak size changed rapidly as a response to environmental changes (Grant and Grant 1989). Beak size is the most variable trait also among the Honeycreepers on the Hawaiian Islands. More than 50 described species of Honeycreepers (subfamily *Drepanidinae*) evolved from a small flock of ancestral finches within some millions of years (Scott et al. 1988). This spectacular example of adaptive radiation has led to a great variety of species with an incredible variety of beak types and beak sizes. From short seed crackers to long curved beaks specialized to reach nectar in long probes (Reding et al. 2009). Beak size is a trait that shows large variation also among the common chaffinch, not only between subspecies but also between populations and islands (Grant 1979). Birds from the Azores have the largest beaks, but also among the Azorean islands there is extensive variation in beak size (Grant 1979; Rodrigues 2012), despite the lack of genetic variation. These examples show that island systems are well suited for studies on speciation and microevolution, and that adaptive radiation can give rise to a large species complex from just a few ancestral colonizers.

## 4.1 Plumage and biometry

The different phenotypes and biometry of the common chaffinch in the present study gives a good reflection of the extant taxonomy. The different subspecies have different coloration, as described by (Grant 1979). The European continental populations are clearly distinguishable from the Macaronesian populations and also from the North African subspecies. The brown back, the strong orange color on the cheeks, and the steel grey color on the head and mantel are unique characters for the European birds. The Macaronesian birds are in general darker blue, and have a paler color on the cheeks and chest. The birds from North Africa share some features with the Macaronesian birds and some with the European, but the ssp. *africana* has a white eye ring that makes them easy distinguishable from the other subspecies of the common chaffinch. The biometrical results from this study correspond well with earlier work (Grant 1979; Dennison and Baker 1991; Rodrigues 2012). The Continental European birds differ substantially from the island birds on several traits. They have longer wings, shorter tarsus, and lower mass than their relatives on the islands. Since the island birds to a large extent are resident these differences are not surprising. It is a well-known phenomenon that resident, birds have shorter wings and larger/bigger bodies than migrating birds (Grant 1965; MacArthur and Wilson 1967; Grant 1979). The populations in the Canaries have differentiated more than populations on the Azores, but are less variable within islands.

## 4.2 Genetic structure and phylogeny

The phylogeny in this study is based on mitochondrial sequences, mainly CO1, but cytb and the control region are also included in the concatenated analyses. MtDNA is widely used in avian taxonomy, and on this level, i.e. species level, it should give a representative picture of the phylogeny and the genetic structure of the chaffinch complex. Since mtDNA evolves fast, it could resolve the closely related populations on some islands.

This is the first complete study with samples from the whole distribution of the Macaronesian chaffinch. In addition, several European populations are included together with the North African subspecies. The genetic results are in general well supported and show good resolution between subspecies and populations. These results correspond well with earlier genetic work (Marshall and Baker 1999; Suárez et al. 2009; Samarasin-Dissanayake 2010).

As for the plumage and the biometry there is a clear differentiation between the Macaronesian Islands and the mainland subspecies. Except for one individual, all the Macaronesian birds are grouped together as a monophyletic clade. For this Atlantic clade, the Azorean samples split out first and divides into two clades. Madeira seems to be the sister group to the Canarian clade which further divides into three groups (Tenerife and La Gomera as one group, El Hierro and La Palma as a second, and Gran Canaria as the third). El Hierro and La Palma are genetically identical on CO1 but are separated when cytb and the control region is included. To investigate this relationship further, more individuals from La Palma and El Hierro were sequenced for cytb and the differentiation between these two populations were retained. So, for these two populations the CO1 sequences do not separate them while cytb does.

On the Azores, the two common haplotypes have no geographic structure. The continental populations cluster together in the NJ-tree and the haplotype network shows that several haplotypes are shared between populations. The Moroccan birds have some unique haplotypes, but also share genes with the European populations. The individuals from Tunisia are caught in an area that is within the distribution of ssp. *africana*. They have two unique haplotypes, but most of the individuals are placed in the Continental clade. However, one individual was found to have a unique and different haplotype from all the others (ind. 38941). In the trees, this individual splits out early and is situated as a sister to the rest of the common chaffinch complex. In the haplotype network, this individual is anchored between the continental and the Atlantic clade together with the Moroccan haplotypes, but the number of mutational steps for this individual is interesting and high compared with the rest of the network. Similar phylogenetic placement has been shown for the ssp. *spodiogenys* (Marshall and Baker 1999): the second North African subspecies that exist in the eastern Tunisia. It is possible that this individual belongs to, or share mtDNA with, this subspecies.

The Macaronesian and the continental branches are well defined and only one bird shows integration of genes from the other clade. This one bird from Faial (Azores) processes a common continental haplotype. After thoroughly checking, it is not likely that this is due to laboratory error, and thus can be interpreted as gene flow between the European continent and the Macaronesian archipelago.

## 4.3 Sperm morphology

The variation in total sperm length across the distribution of the common chaffinch is large and the differences between the two large archipelagos (i.e. the Canaries and the Azores) are striking. Among the Canary Islands there is significant variation between almost all populations, while on the Azores there is no significant variation between any of the islands. The population on Madeira has the longest sperm cells in Macaronesia, similar to the ssp. *africana* who has the longest sperm cells among the continental populations. The total sperm length is continuously declining northwards from Morocco to Norway. The sperm size variation in the Ukrainian population might be artificially low due to difficult samples. Several of the Ukrainian samples had high amount of abnormal sperm, which could be caused by exposure to radioactivity since the sampled birds came from the Chernobyl area. Actually, a study of barn swallows (*Hirundo rustica*) has earlier demonstrated high amounts of abnormal sperm in this area (Møller et al. 2008).

The adjusted $CV_{bm}$ values lies between 0.98 and 2.78 for all populations. According to Lifjeld et al. (2010) this estimations will predict a proportion of extrapair young (EPY) between 46.6% and 14.5%. These estimations imply that the common chaffinch in general is a species with high levels of sperm competition, and a proportion of EPY from average to high.

In general, the sperm measurements reflect the mitochondrial phylogeny. The genetic variation on the Canary Island is consistent with the large sperm morphologic differences. The two common Azorean haplotypes are distributed among the islands without any geographical pattern and there are no significant differences in sperm size between islands.

## 4.4 Sperm competition, gene flow and sperm differentiation

The common rosefinch (*Carpodacus erythrinus*) and the common chaffinch have the longest sperm cells among the *fringillid* species we have studied, i.e. 275 µm and 252 µm, respectively (table 4.1). These two are also the species with the lowest $CV_{bm}$ values (table 4.1), implying that they are both species with high levels of sperm competition. Among the finches there seems to be a trend that the species with low levels of sperm competition have

short sperm and the species with the highest level of sperm competition have the longest. The species with the highest $CV_{bm}$ value and the absolute shortest sperm cells is the European bullfinch (*Pyrrhula pyrrhula*). A recent study comparing the sperm morphology between the European bullfinch and the Azores bullfinch (*Phyrrhula murina*) found that these two geographically separated, but closely related species are identical in total sperm length (Lifjeld et al. In press) (table 4.1). A study on the pied flycatcher (*Ficedula hypoleuca*) showed that between the three studied populations, there were no differences in sperm size (Lifjeld et al. 2012). The pied flycatcher, like the European bullfinch, is a species with low levels of sperm competition, but between these three populations there are reasons to expect some levels of gene flow.

Studies on the red-winged blackbird (*Agelaius phoeniceus*) and the coal tit (*Periparus ater*) showed significant sperm size differences between populations (Lüpold et al. 2011; Schmoll and Kleven 2011). The level of gene flow among populations of both these species is restricted. Further, a comparative study on several populations of barn swallows (*Hirundu rustica*) showed that among five European populations and on East-Mediterranean population there were little variation in sperm size, whereas North American barn swallows differed significantly from the before mentioned populations (Laskemoen et al. in review). Similar to the present study on common chaffinches, the barn swallow study also found high congruence in sperm differentiation and genetic differentiation, with the European and East-Mediterranean subspecies being closely related, whereas the North American subspecies is quite distant (close to 2% pairwise genetic distance) (Dor et al. 2010; Johnsen et al. 2010).

These results indicate that both high levels of sperm competition and low levels of gene flow is required for populations to evolve differences in sperm size. This implication is supported by the results from the mantel test, which showed a positive correlation between genetic distance and sperm morphological distance.

So how do these implications fit in on the Macaronesian common chaffinch complex? First of all, for the common chaffinch there does not seem to be any general trend in which direction the sperm cells evolve. There is large variation between populations, but the total sperm size does not seem to follow any pattern, i.e. birds from Madeira have the longest sperm cells among the Macaronesian Islands, but are geographically situated between the Azores and the Canary Islands. Also, the variation within the Canaries goes in both directions, and there does not seem to be any relationship between sperm size and either geographical placement or

taxonomy. This might imply that total sperm length variation to a large extent is exposed to genetic drift and not directional selection. Further, as earlier stated there is no significant variation in sperm size among the Azorean islands while almost all the Canarian populations are significantly different. The levels of sperm competition are not different between the archipelagos, which can indicate that there are different levels of gene flow between islands within the archipelagos. The Canaries have at least three different morphological phenotypes, while there is one on the Azores. The biometrical measurements show that there is large variation among the Canary Island populations, but there are only small differences among the Azorean Islands (Grant 1979; Rodrigues 2012). The genetic analyses show a similar pattern, with four clades in the Canaries versus one in the Azores. Even though the Azores have two common haplotypes, these are not very different genetically and there is no geographic distributional pattern.

**But why would there be gene flow among the Azorean islands and not in the Canaries?**

The colonization of the Macaronesian Islands is stated to have happened during the last one million years (Marshall and Baker 1999). If this is the case, there should not be any large differences in how long the different archipelagos have been habituated by the common chaffinch, given that these colonization events happened within a short period of time (Samarasin-Dissanayake 2010). The geographical distance between islands within the archipelagos cannot explain the different levels gene flow, since the distance between the Azorean Islands is much greater that between the Canary Islands. One plausible explanation can be different dispersal tendencies. One individual caught in the Azores had a common continental haplotype showing that mixing of genes between the Azores and the European continent happens. This can lead to maintenance of genes coding for migration in the Azorean populations. This could explain the homogeneity that is visible on all measured traits among Azorean Islands.

**Table 4.1.** Comparison of sperm traits between different species in the family Fringillidae. The common chaffinch is among the longest together with the common rosefinch (*Carpodacus* erythrinus). *The common chaffinches used in this table are birds from Norway.

| **common name** | ***genus*** | ***species*** | **head** | **midpiece** | **tail** | **total** | **CVadj_bm** | **CVwm** | **n males** | **Source** |
|---|---|---|---|---|---|---|---|---|---|---|
| American goldfinch | *Carduelis* | *tristis* | 18.1 | 168.4 | 10.8 | 197.3 | 1.67 | 1.16 | 4 | (Kleven et al. 2008) |
| Common redpoll | *Carduelis* | *flammea* | 18.0 | 166.0 | 12.3 | 196.3 | 2.49 | 1.58 | 14 | Laskemoen & Lifjeld unpub data |
| Eurasian siskin | *Carduelis* | *spinus* | 17.7 | 190.0 | 13.1 | 220.8 | 2.36 | 1.13 | 5 | Laskemoen & Lifjeld unpub data |
| European goldfinch | *Carduelis* | *carduelis* | 14.3 | 103.2 | 18.6 | 136.0 | 3.08 | 1.87 | 4 | Laskemoen & Lifjeld unpub data |
| European greenfinch | *Carduelis* | *chloris* | 17.3 | 163.5 | 14.8 | 195.5 | 1.46 | 1.36 | 5 | Laskemoen & Lifjeld unpub data |
| Common rosefinch | *Carpodacus* | *erythrinus* | 15.8 | 249.6 | 9.9 | 275.4 | 1.76 | 1.14 | 11 | Laskemoen & Lifjeld unpub data |
| Brambling | *Fringilla* | *montifringilla* | 18.1 | 184.5 | 10.4 | 213.0 | 2.46 | 1.42 | 14 | Laskemoen & Lifjeld unpub data |
| Common chaffinch* | *Fringilla* | *coelebs* | 16.1 | 225.2 | 10.7 | 252.0 | 2.19 | 1.48 | 39 | This study |
| Common crossbill | *Loxia* | *curvirostra* | 16.3 | 74.3 | 8.6 | 99.2 | 5.12 | 3.64 | 7 | Laskemoen & Lifjeld unpub data |
| Azores bullfinch | *Phyrrhula* | *murina* | 5.6 | NA | 40.0 | 45.6 | 9.62 | 6.07 | 11 | (Lifjeld et al. in press) |
| Eurasian bullfinch | *Pyrrhula* | *pyrrhula* | 5.8 | NA | 40.5 | 46.3 | 8.62 | 7.51 | 13 | (Lifjeld et al. in press) |
| European serin | *Serinus* | *serinus* | 18.6 | 219.2 | 10.2 | 248.0 | 2.25 | 0.97 | 5 | Laskemoen & Lifjeld unpub data |

## 4.5 Colonization route

The colonization history of the common chaffinch is debated and several hypotheses have been proposed. (Grant and Arbore 1980) proposed a two way colonization hypothesis where birds colonized the archipelagos independently from the geographically closest point. So the Canary Islands were colonized by birds from North Africa, whereas the Azores were colonized by birds from the Iberian Peninsula. A one way colonization hypothesis was later presented (Marshall and Baker 1999), stating that the Macaronesian Islands were colonized from only one direction. They argued that birds from North Africa first colonized the Azores, before they moved southwards and colonized Madeira and then the Canary Islands. They conclude that the most likely colonization route within the Canary Islands is from west to east. This one-way hypothesis has also been supported by a later study (Samarasin-Dissanayake 2010), who claims that a European ancestry is more likely, and that the colonization of the Canary Islands might have had the opposite direction, from the east to west. Finally, a mitochondrial and microsatellite study on the Canarian common chaffinches supports the colonization pattern Marshal and Baker (1999) stated, namely that the western islands were colonized first (La Palma) and then eastwards to Gran Canaria (Suárez et al. 2009).

Phylogeographic studies are complex and the possibility for several colonization and re-colonization events between islands make the exact colonization route hard to reconstruct. The two common haplotypes on the Azores might be a result of two independent colonization events. The mitochondrial taxonomy in this study is not enough to conclude on a full colonization history. But, the results from the Neighbor-Joining analysis places the North African populations basally in the tree, thus it might be the ancestral population to the whole common chaffinch complex. From here on there are splits where one branch colonize the Atlantic Islands whereas the other spreads northwards and colonized the European continent. The Pleistocene glacial maximum could have made northern Africa, and partly southern Europe a refuge for many species including the common chaffinch (Griswold and Baker 2002). These types of events could explain the extensive sharing of haplotypes between the European and Moroccan individuals, but also makes a statement of the ancestry difficult. Thus, the decline in haplotype diversity from Morocco and northwards might imply an origin in North Africa. Within the Macaronesian branch the Azorean population splits out first and could be interpreted as being the starting point for the Macaronesian colonization, before

colonizing Madeira and then the Canary Islands. There might have been several colonization and re-colonization events of the different Macaronesian islands. For example some of the founder populations could have consisted of only a few individuals and the genetic variation would then have been limited. Such events can make the colonization history within archipelagos difficult to recreate.

## 4.6 Taxonomic consideration

Several taxonomic revisions have previously been suggested (Marshall and Baker 1999; Suárez et al. 2009). Both these studies acknowledge the population on Gran Canaria as a unique population. The genetic distance between the Macaronesian chaffinch and the continental chaffinch has also been shown. The results in this study supports to a large extent earlier findings, even if there are only mitochondrial genes that are included in this work. But in addition, the sperm morphological measurements bring a new element into the discussion. The most striking deviation from the current taxonomy is the unique population from Gran Canaria. Currently, this population belongs to ssp. *canariensis*, together with the populations from Tenerife and La Gomera. However the present study has demonstrated that the Gran Canarian population is clearly different from the two others on all measured traits. They have a unique mitochondrial haplotype, a different biometry, and the total sperm size is significantly different from all the other Canarian populations. There might also be a difference in plumage, but this will have to be studied more thoroughly in later work. The maintenances of the current taxonomy, regarding the two western Canarian populations are supported. The birds from Madeira and the Azoreans are both monophyletic groups. Further, the pairwise genetic distance between the Macaronesian populations and the continental populations exceeds 2.5% (appendix 6.), a differentiation that is similar to what is found between described species of closely related passerines (Dietzen et al. 2003). The relationship between the continental populations is messy. The North African birds are clearly morphologically different from the European populations, but genetically there is extensive sharing of haplotypes between these two continents. Several expansions and retractions of populations caused by ice ages could have mixed the population and created this pattern (Griswold and Baker 2002).

As in other island systems, the classification of species is demanding, and the different species concepts can give different results. The phylogenetic species concept (PSC) states that the smallest monophyletic taxonomic unit should be treated as a species (Cracraft 1983; Davis and Nixon 1992; Quentin et al. 2000). The implications of this concept on the common chaffinch complex will be extensive. In the strictest sense, the use of this concept would lead to six new species only for the Macaronesian islands. All of the Macaronesian subspecies should be elevated to species level, implicating new endemic species on both the Azores and on Madeira. Further, on the Canary Islands, the ssp. *canariensis* would be split, and both the population on Gran Canaria and the populations on Tenerife/La Gomera should be classified as unique species. The two western populations on La Palma and El Hierro would also be unique species.

By using the biological species concept (BSC) (Mayr 1963) the implications would be more conservative. The reproductive isolation that is important to separate between species according to the BSC, creates difficulties. For a system with several remote isolated islands where the populations rarely or never meet, a question of reproductive isolation would be of hypothetical character. It is well known that chaffinches in captivity are possible to breed with other subspecies or even other finch species, but this never or rarely happens in the wild. In natural populations there might exist premating barriers, like differences in song, plumage, physiology, and/or behavior that prevents hybridization. But, for occasionally hybridizing populations the barriers might be on a postmating level, where sperm competition can be an important mechanism. High sperm competition within isolated populations would select for a sperm size closest to the population optimum. Selection for different sperm size optimums in different populations would then also be selection against hybridization. When this optimum is significantly different between two populations, it is likely that an interbreeding of individuals from these populations would encounter a decrease in fertilization success. For example, the large difference in sperm size between the neighboring populations on Tenerife and Gran Canaria (> 20 µm) could affect the fertilizing success, if individuals from these two populations met and copulated. Such a postmating mechanism could then be a predictor of reproductive barriers without observing hybridization between two populations in the wild. However, to split the ssp. *canariensis* in two and acknowledge the population on Gran Canaria as a separate subspecies is well supported. Further, to split the common chaffinch into one continental and one Macaronesian species might also be argued according to the BSC, depending on the definition of a species. The variation between the Macaronesian and the

continental subspecies are extensive and the level of gene flow between them seems restricted. The BSC are fundamental in avian taxonomy, but sperm morphological characters and differences in sperm morphology between populations might be considered as a reproductive barrier and taken into account when the common chaffinch complex is revised.

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# 6 Appendix

**Appendix 1**. A complete list over all the individuals that are used for genetic analyzes.

| species_name | processID | recordID | institution_storing | country | province | date | Haplotype |
|---|---|---|---|---|---|---|---|
| *Fringilla montifringilla* | KBPBK037-08 | 822679 | Burke Museum of Natural History and Culture | Russia | Murmansk | 14-Jul-1990 | NA |
| *Fringilla montifringilla* | KBPBK073-08 | 822715 | Burke Museum of Natural History and Culture | Russia | Magadanskaya Oblast | 16-Jul-1988 | NA |
| *Fringilla montifringilla* | KBPBK083-08 | 822725 | Burke Museum of Natural History and Culture | Russia | Primor'ye | 17-Sep-1990 | NA |
| *Fringilla montifringilla* | KBPBK150-08 | 822792 | Burke Museum of Natural History and Culture | Russia | Buryatiya Respublika | 22-Jun-1989 | NA |
| *Fringilla montifringilla* | BISE058-07 | 634153 | Swedish Museum of Natural History | Sweden | Norrbotten | 27-Jun-1998 | NA |
| *Fringilla montifringilla* | BON018-06 | 297980 | University of Oslo, Natural History Museum | Norway | Finnmark | 04-Aug-1966 | NA |
| *Fringilla montifringilla* | BON024-06 | 297986 | University of Oslo, Natural History Museum | Norway | Finnmark | 05-Aug-1966 | NA |
| *Fringilla montifringilla* | KBPBK040-08 | 822682 | Burke Museum of Natural History and Culture | Russia | Tyumen Region | 08-Jun-1992 | NA |
| *Fringilla montifringilla* | KBPBK182-08 | 822824 | Burke Museum of Natural History and Culture | Russia | Tuva | 26-Jun-1995 | NA |
| *Fringilla montifringilla* | KBPBK233-08 | 822875 | Burke Museum of Natural History and Culture | Russia | Irkutsk Region | 23-Jun-2000 | NA |
| *Fringilla montifringilla* | KBPBK255-08 | 822897 | Burke Museum of Natural History and Culture | Russia | Kamchatka | 20-Jul-1998 | NA |
| *Fringilla montifringilla* | KBPBK295-08 | 822937 | Burke Museum of Natural History and Culture | Russia | Yamal-Nenets AP | 30-May-1993 | NA |
| *Fringilla montifringilla* | KBPBK333-08 | 822975 | Burke Museum of Natural History and Culture | Russia | Chukot AP | 28-Jun-1999 | NA |
| *Fringilla montifringilla* | TZBNA219-03 | 3157 | Royal Ontario Museum | United States | Alaska | | |
| *Fringilla montifringilla* | BISE266-08 | 816765 | Swedish Museum of Natural History | Sweden | Vastra Gotaland | 12-Dec-2001 | NA |
| *Fringilla montifringilla* | BOTW119-04 | 11898 | National Museum of Natural History, Smithsonian Institution | Sweden | Lappland | | |
| | | | | | | | |
| *Fringilla teydea* | BONMA152-11 | NHMO-32803 | University of Oslo, Natural History Museum | Spain | Tenerife | 30-Apr-2010 | NA |
| *Fringilla teydea* | BONMA366-12 | NHMO-32729 | University of Oslo, Natural History Museum | Spain | Tenerife | 26-Apr-2010 | NA |
| *Fringilla teydea* | BONMA150-11 | NHMO-32791 | University of Oslo, Natural History Museum | Spain | Tenerife | 29-Apr-2010 | NA |
| *Fringilla teydea* | BONMA357-12 | NHMO-26444 | University of Oslo, Natural History Museum | Spain | Tenerife | 23-Mar-2009 | NA |
| *Fringilla teydea* | BONMA101-11 | NHMO-26446 | University of Oslo, Natural History Museum | Spain | Tenerife | 23-Mar-2009 | NA |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *Fringilla coelebs* | BISE265-08 | 816763 | Swedish Museum of Natural History | Sweden | Stockholm | 03-Aug-1995 | H_17 |
| *Fringilla coelebs* | KBPZM008-06 | 281886 | Zoological Museum of Moscow University | Russia | Kaliningradskaya Oblast | | H_17 |
| *Fringilla coelebs* | KBPZM015-06 | 281893 | Zoological Museum of Moscow University | Russia | Kaliningradskaya Oblast | | H_17 |
| *Fringilla coelebs* | BONMA224-11 | NHMO-34128 | University of Oslo, Natural History Museum | Portugal | Azores, Faial | 16-Jul-2008 | H_17 |
| *Fringilla coelebs* | BONMA118-11 | NHMO-31824 | University of Oslo, Natural History Museum | Spain | Alamillo | 19-Dec-2006 | H_17 |
| *Fringilla coelebs* | BONMA119-11 | NHMO-31825 | University of Oslo, Natural History Museum | Spain | Alamillo | 19-Dec-2006 | H_17 |
| *Fringilla coelebs* | BONMA298-11 | NHMO-35358 | University of Oslo, Natural History Museum | Spain | Collserola | 02-May-2011 | H_18 |
| *Fringilla coelebs* | BONMO004-09 | SAS-006 | University of Oslo, Natural History Museum | Morocco | Azrou | 12-May-2008 | H_17 |
| *Fringilla coelebs* | KBPBK025-08 | 822667 | Burke Museum of Natural History and Culture | Russia | Sverdlovsk Region | | H_17 |
| *Fringilla coelebs* | BONPA008-10 | NHMO-33099 | University of Oslo, Natural History Museum | Spain | Cecebre | 28-May-2010 | H_22 |
| *Fringilla coelebs* | BON297-07 | 573216 | University of Oslo, Natural History Museum | Norway | Telemark | | H_19 |
| *Fringilla coelebs* | BONMA183-11 | NHMO-34010 | University of Oslo, Natural History Museum | Morocco | Ifrane, Middle Atlas | 23-Mar-2010 | H_15 |
| *Fringilla coelebs* | BONMA185-11 | NHMO-34015 | University of Oslo, Natural History Museum | Morocco | Ifrane, Middle Atlas | 23-Mar-2010 | H_15 |
| *Fringilla coelebs* | BONMA184-11 | NHMO-34011 | University of Oslo, Natural History Museum | Morocco | Ifrane, Middle Atlas | 23-Mar-2010 | H_15 |
| *Fringilla coelebs* | BONMA186-11 | NHMO-34020 | University of Oslo, Natural History Museum | Morocco | Ifrane, Middle Atlas | 23-Mar-2010 | H_16 |
| *Fringilla coelebs* | BISE006-07 | 634101 | Swedish Museum of Natural History | Sweden | Stockholm | | H_15 |
| *Fringilla coelebs* | BON080-06 | 298042 | University of Oslo, Natural History Museum | Norway | Telemark | | H_15 |
| *Fringilla coelebs* | BONMO035-10 | NHMO-24260 | University of Oslo, Natural History Museum | Morocco | Azrou | 13-May-2008 | H_21 |
| *Fringilla coelebs* | BONMO036-10 | NHMO-24261 | University of Oslo, Natural History Museum | Morocco | Azrou | 13-May-2008 | H_21 |
| *Fringilla coelebs* | BONMO037-10 | NHMO-24262 | University of Oslo, Natural History Museum | Morocco | Azrou | 13-May-2008 | H_21 |
| *Fringilla coelebs* | BONMO005-09 | SAS-007 | University of Oslo, Natural History Museum | Morocco | Azrou | 12-May-2008 | H_20 |
| *Fringilla coelebs* | BONMA229-11 | NHMO-34133 | University of Oslo, Natural History Museum | Portugal | Azores, Graciosa | 29-Aug-2008 | H_1 |
| *Fringilla coelebs* | BONMA235-11 | NHMO-34139 | University of Oslo, Natural History Museum | Portugal | Azores, Sao Jorge | 02-Jul-2008 | H_1 |
| *Fringilla coelebs* | BONMA230-11 | NHMO-34134 | University of Oslo, Natural History Museum | Portugal | Azores, Graciosa | 30-Aug-2008 | H_1 |
| *Fringilla coelebs* | BONMA231-11 | NHMO-34135 | University of Oslo, Natural History Museum | Portugal | Azores, Pico | 07-Jul-2008 | H_1 |
| *Fringilla coelebs* | BONMA242-11 | NHMO-34149 | University of Oslo, Natural History Museum | Portugal | Azores, Terceira | 01-Sep-2008 | H_1 |
| *Fringilla coelebs* | BONMA228-11 | NHMO-34132 | University of Oslo, Natural History Museum | Portugal | Azores, Graciosa | 29-Aug-2008 | H_1 |
| *Fringilla coelebs* | BONMA226-11 | NHMO-34130 | University of Oslo, Natural History Museum | Portugal | Azores, Flores | 24-Sep-2008 | H_1 |
| *Fringilla coelebs* | BONMA240-11 | NHMO-34147 | University of Oslo, Natural History Museum | Portugal | Azores, Terceira | 26-Aug-2008 | H_1 |

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|---|---|---|---|---|---|---|---|
| *Fringilla coelebs* | BONMA237-11 | NHMO-34141 | University of Oslo, Natural History Museum | Portugal | Azores, Sao Maria | 28-May-2008 | H_1 |
| *Fringilla coelebs* | BONMA233-11 | NHMO-34137 | University of Oslo, Natural History Museum | Portugal | Azores, Pico | 11-Jul-2008 | H_1 |
| *Fringilla coelebs* | BONMA031-10 | NHMO-32579 | University of Oslo, Natural History Museum | Portugal | Azores, San Miguel | 01-May-2010 | H_1 |
| *Fringilla coelebs* | BONMA029-10 | NHMO-32570 | University of Oslo, Natural History Museum | Portugal | Azores, San Miguel | 30-Apr-2010 | H_1 |
| *Fringilla coelebs* | BONMA030-10 | NHMO-32573 | University of Oslo, Natural History Museum | Portugal | Azores, San Miguel | 29-Apr-2010 | H_1 |
| *Fringilla coelebs* | BONMA234-11 | NHMO-34138 | University of Oslo, Natural History Museum | Portugal | Azores, Sao Jorge | 01-Jul-2008 | H_1 |
| *Fringilla coelebs* | BONMA027-10 | NHMO-32566 | University of Oslo, Natural History Museum | Portugal | Azores, San Miguel | 29-Apr-2010 | H_1 |
| *Fringilla coelebs* | BONMA219-11 | NHMO-34123 | University of Oslo, Natural History Museum | Portugal | Azores, Corvo | 18-Sep-2008 | H_6 |
| *Fringilla coelebs* | BONMA232-11 | NHMO-34136 | University of Oslo, Natural History Museum | Portugal | Azores, Pico | 08-Jul-2008 | H_6 |
| *Fringilla coelebs* | BONMA227-11 | NHMO-34131 | University of Oslo, Natural History Museum | Portugal | Azores, Flores | 25-Sep-2008 | H_6 |
| *Fringilla coelebs* | BONMA222-11 | NHMO-34126 | University of Oslo, Natural History Museum | Portugal | Azores, Faial | 13-Jul-2008 | H_6 |
| *Fringilla coelebs* | BONMA241-11 | NHMO-34148 | University of Oslo, Natural History Museum | Portugal | Azores, Terceira | 27-Aug-2008 | H_6 |
| *Fringilla coelebs* | BONMA220-11 | NHMO-34124 | University of Oslo, Natural History Museum | Portugal | Azores, Corvo | 19-Sep-2008 | H_6 |
| *Fringilla coelebs* | BONMA236-11 | NHMO-34140 | University of Oslo, Natural History Museum | Portugal | Azores, Sao Jorge | 03-Jul-2008 | H_6 |
| *Fringilla coelebs* | BONMA225-11 | NHMO-34129 | University of Oslo, Natural History Museum | Portugal | Azores, Flores | 23-Sep-2008 | H_6 |
| *Fringilla coelebs* | BONMA223-11 | NHMO-34127 | University of Oslo, Natural History Museum | Portugal | Azores, Faial | 14-Jul-2008 | H_6 |
| *Fringilla coelebs* | BONMA221-11 | NHMO-34125 | University of Oslo, Natural History Museum | Portugal | Azores, Corvo | 20-Sep-2008 | H_6 |
| *Fringilla coelebs* | BONMA028-10 | NHMO-32567 | University of Oslo, Natural History Museum | Portugal | Azores, San Miguel | 29-Apr-2010 | H_6 |
| *Fringilla coelebs* | BONMA239-11 | NHMO-34143 | University of Oslo, Natural History Museum | Portugal | Azores, Sao Maria | 29-May-2008 | H_7 |
| *Fringilla coelebs* | BONMA238-11 | NHMO-34142 | University of Oslo, Natural History Museum | Portugal | Azores, Sao Maria | 29-May-2008 | H_7 |
| *Fringilla coelebs* | BONMA037-10 | NHMO-26537 | University of Oslo, Natural History Museum | Spain | Gran Canaria | 02-Apr-2009 | H_8 |
| *Fringilla coelebs* | BONMA244-11 | NHMO-34633 | University of Oslo, Natural History Museum | Spain | Gran Canaria | 13-Apr-2011 | H_8 |
| *Fringilla coelebs* | BONMA246-11 | NHMO-34635 | University of Oslo, Natural History Museum | Spain | Gran Canaria | 13-Apr-2011 | H_8 |
| *Fringilla coelebs* | BONMA250-11 | NHMO-34825 | University of Oslo, Natural History Museum | Spain | Gran Canaria | 01-Mar-2011 | H_8 |
| *Fringilla coelebs* | BONMA245-11 | NHMO-34634 | University of Oslo, Natural History Museum | Spain | Gran Canaria | 13-Apr-2011 | H_8 |
| *Fringilla coelebs* | BONMA045-10 | NHMO-26479 | University of Oslo, Natural History Museum | Spain | Tenerife | 26-Mar-2009 | H_11 |
| *Fringilla coelebs* | BONMA258-11 | NHMO-35107 | University of Oslo, Natural History Museum | Spain | La Gomera | 28-Apr-2011 | H_11 |
| *Fringilla coelebs* | BONMA106-11 | NHMO-26486 | University of Oslo, Natural History Museum | Spain | Tenerife | 26-Mar-2009 | H_11 |
| *Fringilla coelebs* | BONMA043-10 | NHMO-26462 | University of Oslo, Natural History Museum | Spain | Tenerife | 24-Mar-2009 | H_9 |
| *Fringilla coelebs* | BONMA104-11 | NHMO-26484 | University of Oslo, Natural History Museum | Spain | Tenerife | 26-Mar-2009 | H_9 |

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|---|---|---|---|---|---|---|---|
| *Fringilla coelebs* | BONMA105-11 | NHMO-26485 | University of Oslo, Natural History Museum | Spain | Tenerife | 26-Mar-2009 | H_9 |
| *Fringilla coelebs* | BONMA044-10 | NHMO-26463 | University of Oslo, Natural History Museum | Spain | Tenerife | 24-Mar-2009 | H_10 |
| *Fringilla coelebs* | BONMA269-11 | NHMO-35128 | University of Oslo, Natural History Museum | Spain | La Gomera | 29-Apr-2011 | H_10 |
| *Fringilla coelebs* | BONMA271-11 | NHMO-35133 | University of Oslo, Natural History Museum | Spain | La Gomera | 29-Apr-2011 | H_10 |
| *Fringilla coelebs* | BONMA116-11 | NHMO-26567 | University of Oslo, Natural History Museum | Spain | Tenerife | 07-Apr-2009 | H_10 |
| *Fringilla coelebs* | BONMA273-11 | NHMO-35151 | University of Oslo, Natural History Museum | Spain | La Gomera | 29-Apr-2011 | H_10 |
| *Fringilla coelebs* | BONMA032-10 | NHMO-32638 | University of Oslo, Natural History Museum | Spain | El Hierro | 18-Apr-2010 | H_10 |
| *Fringilla coelebs* | BONMA033-10 | NHMO-32645 | University of Oslo, Natural History Museum | Spain | El Hierro | 19-Apr-2010 | H_10 |
| *Fringilla coelebs* | BONMA034-10 | NHMO-32646 | University of Oslo, Natural History Museum | Spain | El Hierro | 19-Apr-2010 | H_10 |
| *Fringilla coelebs* | BONMA035-10 | NHMO-32647 | University of Oslo, Natural History Museum | Spain | El Hierro | 19-Apr-2010 | H_10 |
| *Fringilla coelebs* | BONMA036-10 | NHMO-32654 | University of Oslo, Natural History Museum | Spain | El Hierro | 19-Apr-2010 | H_10 |
| *Fringilla coelebs* | BONMA038-10 | NHMO-32837 | University of Oslo, Natural History Museum | Spain | La Palma | 03-May-2010 | H_10 |
| *Fringilla coelebs* | BONMA039-10 | NHMO-32844 | University of Oslo, Natural History Museum | Spain | La Palma | 04-May-2010 | H_10 |
| *Fringilla coelebs* | BONMA040-10 | NHMO-32846 | University of Oslo, Natural History Museum | Spain | La Palma | 04-May-2010 | H_10 |
| *Fringilla coelebs* | BONMA041-10 | NHMO-32850 | University of Oslo, Natural History Museum | Spain | La Palma | 04-May-2010 | H_10 |
| *Fringilla coelebs* | BONMA042-10 | NHMO-32851 | University of Oslo, Natural History Museum | Spain | La Palma | 04-May-2010 | H_10 |
| *Fringilla coelebs* | BONMA278-11 | NHMO-35174 | University of Oslo, Natural History Museum | Portugal | Madeira | 03-May-2011 | H_14 |
| *Fringilla coelebs* | BONMA282-11 | NHMO-35186 | University of Oslo, Natural History Museum | Portugal | Madeira | 04-May-2011 | H_13 |
| *Fringilla coelebs* | BONMA279-11 | NHMO-35179 | University of Oslo, Natural History Museum | Portugal | Madeira | 04-May-2011 | H_13 |
| *Fringilla coelebs* | BONMA281-11 | NHMO-35185 | University of Oslo, Natural History Museum | Portugal | Madeira | 04-May-2011 | H_13 |
| *Fringilla coelebs* | BONMO046-12 | NHMO-38940 | University of Oslo, Natural History Museum | Tunisia | Ain Soltane, 333 | 09-May-2012 | H_2 |
| *Fringilla coelebs* | BONMO047-12 | NHMO-38941 | University of Oslo, Natural History Museum | Tunisia | Ain Soltane, 349 | 12-May-2012 | H_5 |
| *Fringilla coelebs* | BONMO048-12 | NHMO-38942 | University of Oslo, Natural History Museum | Tunisia | Ain Soltane, 364 | 15-May-2012 | H_4 |
| *Fringilla coelebs* | BONMO049-12 | NHMO-38943 | University of Oslo, Natural History Museum | Tunisia | Ain Soltane, 373 | 15-May-2012 | H_4 |
| *Fringilla coelebs* | BONMO050-12 | NHMO-38944 | University of Oslo, Natural History Museum | Tunisia | Ain Soltane, 389 | 18-May-2012 | H_3 |

**Appendix 2**. The table illustrates the variable sites at the 22 different haplotypes.

| | Position | | |

**Appendix 3.** Pairwise t-test comparison of the Canarian populations. Gran Canaria is significantly different from all other populations.

| | **Gran Canaria** | **Tenerife** | **La Gomera** | **El Hierro** | **La Palma** |
|---|---|---|---|---|---|
| **Tenerife** | < 0.001 | | | | |
| **La Gomera** | < 0.001 | 0.1632 | | | |
| **El Hierro** | < 0.001 | < 0.001 | 1 | | |
| **La Palma** | 0.0126 | < 0.001 | < 0.001 | 0.0220 | |
| **Madeira** | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 |

**Appendix 4.** For the between group distances, the groups do not reflect the current taxonomy. For the Canary Islands, the distances are calculated between islands and not subpopulations, since Gran Canaria clearly are genetically different from the two others (Tenerife and La Gomera). The islands on Azores are all lumped together since there are no differences between them. The European populations are also lumped together, partly of the same reason, but also that the sample sizes are variable. The Kimura 2-parameter model is used for the calculations.

| | **Azores** | **Tunisia** | **Gran Canaria** | **Tenerife** | **La Gomera** | **El Hierro** | **La Palma** | **Madeira** | **Morocco** |
|---|---|---|---|---|---|---|---|---|---|
| **Tunisia** | 0.031 | | | | | | | | |
| **Gran Canaria** | 0.024 | 0.031 | | | | | | | |
| **Tenerife** | 0.024 | 0.032 | 0.014 | | | | | | |
| **La Gomera** | 0.023 | 0.031 | 0.013 | 0.002 | | | | | |
| **El Hierro** | 0.022 | 0.033 | 0.012 | 0.012 | 0.012 | | | | |
| **La Palma** | 0.022 | 0.033 | 0.012 | 0.012 | 0.012 | 0.000 | | | |
| **Madeira** | 0.025 | 0.033 | 0.021 | 0.021 | 0.020 | 0.016 | 0.016 | | |
| **Morocco** | 0.025 | 0.015 | 0.026 | 0.025 | 0.024 | 0.026 | 0.026 | 0.026 | |
| **Europe** | 0.028 | 0.010 | 0.027 | 0.027 | 0.026 | 0.028 | 0.028 | 0.029 | 0.007 |

**Appendix 5.** All the Canary Island populations are lumped together, the same for the Azores. Kimura 2-paramameter model are used for the calculations of the between group genetic distance.

| | **Azores** | **Canary Islands** |
|---|---|---|
| **Canary Islands** | 0.022 | |
| **Madeira** | 0.022 | 0.018 |

**Appendix 6.** The genetic distance between all the Macaronesian populations combined and all the Continental populations combined show a distance = 0.027. Kimura 2-parameter model is also here used for the calculation of the difference.

| | **Continental** |
|---|---|
| **Macaronesia** | 0.027 |